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Synthesis of (±)-brevioxime and (±)-puraquinonic acid and studies on peptide ligation and acyl transfer

©

by

Soleiman Hisaindee

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

DEPARTMENT OF CHEMISTRY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Synthesis of (±)-brevioxime and (±)-puraquinonic acid and studies on peptide ligation and acyl transfer** submitted by **Soleiman Hisaindee** in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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To my family

ABSTRACT

The first Chapter of this thesis describes the synthesis of (±)-brevioxime. This fungal metabolite inhibits juvenile hormone biosynthesis and therefore constitutes a potential lead compound for development of novel insecticides. The most conspicuous feature of brevioxime is its hitherto unknown heterobicyclic core structure. Herein are described exploratory studies that led to the construction of this unique system by an acid-catalyzed double condensation of a β-ketoamide bearing a masked aldehyde. The methodology was applied first to the preparation of two model compounds and later to (±)-brevioxime. The route should be amenable to the preparation of analogs. The results of this work have been published (*Chem. Commun.* **1999**, 2251; *J. Org. Chem.* **2000**, *65*, 4923).

The second part of this thesis deals with synthetic studies on puraquinonic acid, a fungal metabolite that induces differentiation in leukemic HL-60 cells. Puraquinonic acid contains an asymmetric quaternary center, and the features of the molecule that are responsible for this asymmetry are far removed from it. Two routes for the synthesis of (±)-puraquinonic acid were developed, and approaches to the synthesis of the optically pure natural product were also explored. The syntheses of (±)-puraquinonic acid have been published (*J. Org. Chem.* **2001**, *66*, 954; *Tetrahedron Lett.* **2001**, *42*, 2253).

The third part of this thesis describes studies towards peptide segment coupling by prior ligation and proximity-induced intramolecular acyl transfer. Native chemical ligation is one of the emerging techniques for the synthesis of large proteins, both natural and unnatural, but the current approaches impose a severe restriction in that the *N*-terminus of one of he segments to be ligated has to be cysteine or glycine. The present studies aim at the development of a general method that will allow peptides to be ligated regardless of the identity of the amino acid at the *N*-terminus. Thus, specially derivatized amino acids that contain a cleavable template have been synthesized and were coupled to the *N*-terminus of short test segments. The template was then elaborated into a system that will, eventually, allow for the capture of another peptide segment via its *C*-terminus.

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List of abbreviations

Ac	acetyl
AIBN	2,2'-azobisisobutyronitrile
iso-Amyl	$Me_2CHCH_2CH_2$
Ar	aryl
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bu	butyl
t-Bu	*tert*-butyl
calcd	calculated
Cbz	benzyloxycarbonyl
DCC	*N,N*-dicyclohexylcarbodiimide
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DHP	dihydropyran
DIBAL-H	diisobutylaluminum hydride
DMAP	4-(dimethylamino)pyridine
DME	ethylene glycol dimethyl ether
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EDCI	*N*-(3-dimethylamino)propyl-*N*-ethylcarbodiimde
Et	ethyl
Fmoc	9-fluorenylmethoxycarbonyl
h	hour(s)
HBTU	*O*-benzotriazol-1-yl-*N,N,N',N'* tetramethyluronium hexafluorophosphate
Hz	hertz

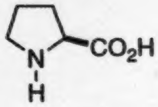
Im	imidazoyl
LAH	lithium aluminum hydride
LDA	lithium diisopropylamide
Me	methyl
min	minute(s)
mp	melting point
Ms	methanesulfonyl
MS	mass spectrometry
NBS	*N*-bromosuccinimide
NMO	4-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser enhancement
Oxone	potassium peroxymonosulfate ($2KHSO_5.KHSO_4.K_2SO_4$)
PCC	pyridinium chlorochromate
Pg	protecting group
Ph	phenyl
PPTS	pyridinium *p*-toluenesulfonic acid
SAMP	(*S*)-(-)-1-amino-2-(methoxymethyl)pyrrolidine
TBAF	tetrabutylammonium fluoride
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TPAP	tetra-*n*-propylammonium perruthenate
Troc	2,2,2-trichloroethoxycarbonyl
Ts	*p*-toluenesulfonyl

TsOH *p*-toluenesulfonic acid

Amino acid abbreviations:

Three letter symbol	**Amino acid**	**One letter symbol**	**Side chain**
Ala	Alanine	A	CH_3
Arg	Arginine	R	$(CH_2)_3NHC(=NH)NH$
Asn	Asparagine	N	CH_2CONH_2
Asp	Aspartic acid	D	CH_2CO_2H
Cys	Cysteine	C	CH_2SH
Gln	Glutamine	Q	$(CH_2)_2CONH_2$
Glu	Glutamic acid	E	$(CH_2)_2CO_2H$
Gly	Glycine	G	H
His	Histidine	H	CH_2(4-imidazolyl)
Ile	Isoleucine	I	$CH(CH_3)CH_2CH_3$
Leu	Leucine	L	$CH_2CH(CH_3)_2$
Lys	Lysine	K	$(CH_2)_4NH_2$
Met	Methionine	M	$(CH_2)_2SCH_3$
Phe	Phenylalanine	F	CH_2Ph
Pro	Proline	P	See below
Ser	Serine	S	CH_2OH
Thr	Threonine	T	$CH(CH_3)OH$
Trp	Tryptophan	W	CH_2(3-indolyl)
Tyr	Tyrosine	Y	CH_2(4-hydroxyphenyl)
Val	Valine	V	$CH(CH_3)_2$

Proline:



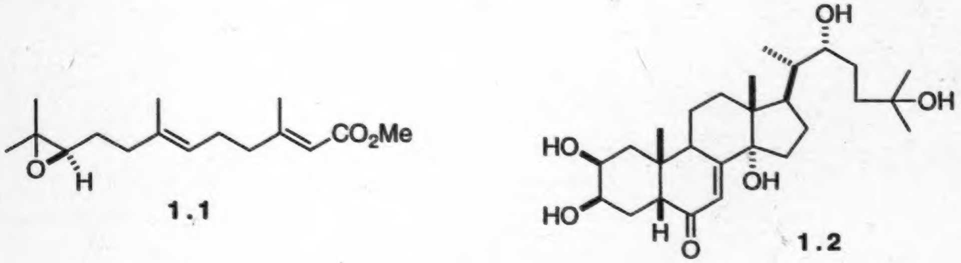
SYNTHESIS OF (±)-BREVIOXIME AND (±)-PURAQUINONIC ACID AND STUDIES ON PEPTIDE LIGATION AND ACYL TRANSFER

CHAPTER 1

SYNTHESIS OF (±)-BREVIOXIME

Introduction

Insect growth and development is controlled by hormones, and substances that inhibit the formation of these hormones represent, in principle, an attractive method for pest control.[1] Well-known examples of these hormones are juvenile hormones, such as juvenile hormone III (**1.1**) (Scheme 1), and ecdysone (**1.2**).[1]



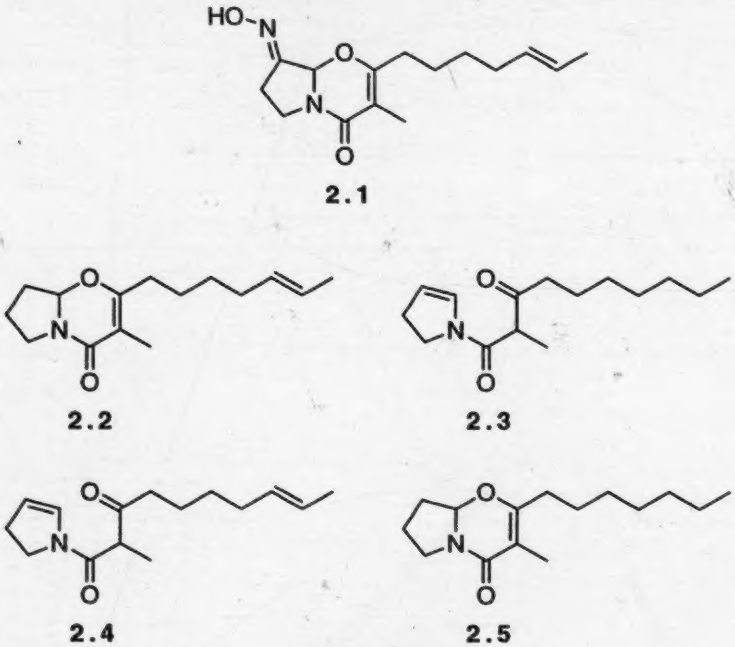
1.1

1.2

Scheme 1

The first part of this Thesis deals with the synthesis of brevioxime (**2.1**), which inhibits the formation of one of these important insect hormones – juvenile hormone III.

Brevioxime was isolated[2,3] from a strain of the fungus *Penicillium brevicompactum*, and its structure was reported a few years ago.[2] The substance was found to be a potent inhibitor of juvenile hormone III biosynthesis[2,3] and it appears to block those enzymatic steps of the isoprenoid pathway that are specific for insects.[3] These properties make it a potential lead compound for the development of



Scheme 2

insecticides. The related compounds **2.2**,[4] **2.3**,[5] and **2.4**[4] have also been isolated from the same fungus, and their biological properties examined. Compounds **2.3** and **2.4** both inhibit formation of juvenile hormone, but the mode of action of **2.2**, which is also an insecticide, does not appear to have been established.

The structure of brevioxime is an unusual one and, although a number of benzo-fused substances containing a similar ring system are known, those lacking a fused benzene ring are rare.[6,7] The only other unsaturated examples we know of are compounds made in this laboratory and described below, as well as model compounds prepared[4,5] by the discoverers of brevioxime.

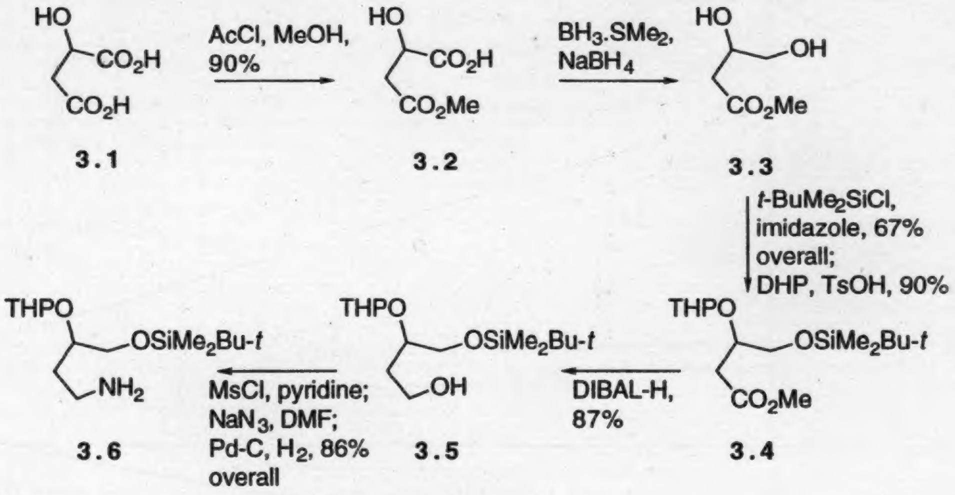
Other syntheses of brevioxime

Apart from our own synthesis,[8] which is discussed in the next section, three other syntheses of brevioxime have been reported, as well as the preparation of some simple model compounds.

Kitahara's synthesis

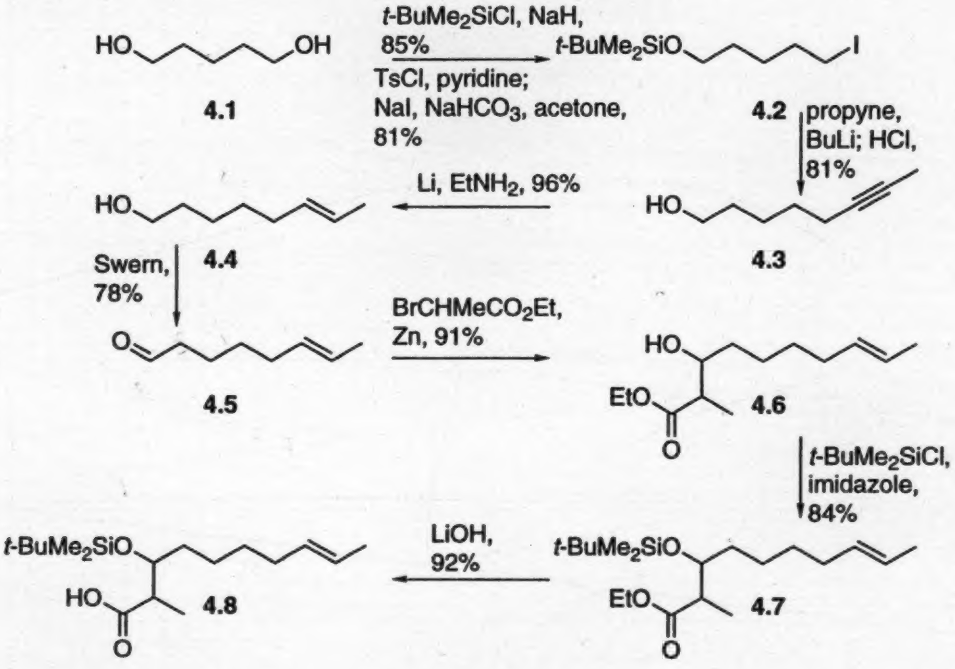
Nishimura and Kitahara reported[9] a synthesis that is very similar in conception to our route. Their work actually represents the first synthesis of brevioxime, as the receipt date for their manuscript is 1 May 1999, while that for our work is 10 August 1999. However, the Kitahara paper did not appear until the following year, and we were unaware of it even when we later submitted our own full paper. We do not know the reason for the apparent delay in publishing the Japanese work.

Nishimura and Kitahara assembled racemic brevioxime from two subunits **3.6** and **4.8**. The former was made (Scheme 3) from (±)-malic acid (**3.1**) by esterification and borane reduction of the remaining carboxyl (**3.1** → **3.2** → **3.3**). Selective silylation of the primary hydroxyl of **3.3** and protection of the remaining secondary hydroxyl as its THP ether gave **3.4**. This was reduced to a primary alcohol (**3.4** → **3.5**), and the hydroxyl was replaced by an amino group,


HO
CO₂H
CO₂H
3.1
AcCl, MeOH, 90%
HO
CO₂H
CO₂Me
3.2
BH₃.SMe₂, NaBH₄
HO
OH
CO₂Me
3.3
t-BuMe₂SiCl, imidazole, 67% overall;
DHP, TsOH, 90%
THPO
OSiMe₂Bu-t
CO₂Me
3.4
DIBAL-H, 87%
THPO
OSiMe₂Bu-t
OH
3.5
MsCl, pyridine;
NaN₃, DMF;
Pd-C, H₂, 86% overall
THPO
OSiMe₂Bu-t
NH₂
3.6

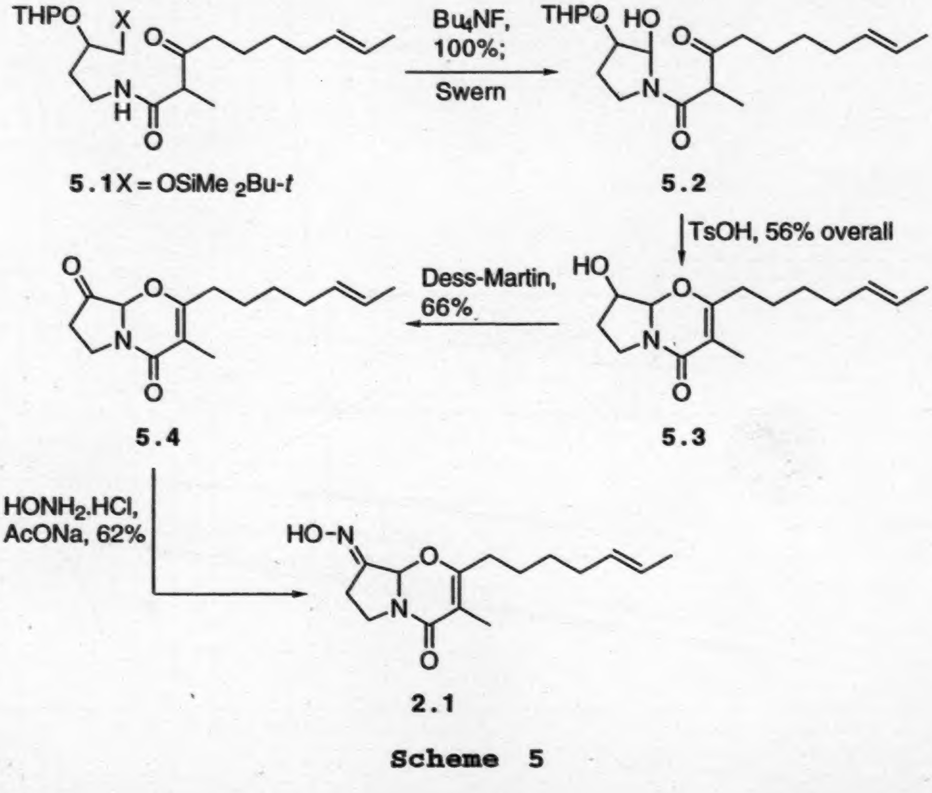
Scheme 3

using the classical sequence: mesylation, displacement with azide ion, and hydrogenation. All steps leading to the final amine **3.6** worked in acceptable yields.


HO
OH
4.1
t-BuMe₂SiCl, NaH, 85%
TsCl, pyridine;
NaI, NaHCO₃, acetone, 81%
t-BuMe₂SiO
I
4.2
propyne, BuLi; HCl, 81%
HO
4.3
Li, EtNH₂, 96%
HO
4.4
Swern, 78%
O
4.5
BrCHMeCO₂Et, Zn, 91%
HO
EtO
O
4.6
t-BuMe₂SiCl, imidazole, 84%
t-BuMe₂SiO
EtO
O
4.7
LiOH, 92%
t-BuMe₂SiO
HO
O
4.8

Scheme 4

The other subunit was acid **4.8**, which was prepared as shown in Scheme 4. The pentanediol **4.1** was monosilylated and the remaining hydroxyl was replaced by iodine via tosylate displacement. The iodide, in turn was displaced, using the acetylide generated from propyne, and removal of the silyl protecting group then gave acetylenic alcohol **4.3**. Dissolving metal reduction converted the triple bond into an *E*-double bond (**4.3** → **4.4**), and the resulting alcohol **4.4** was oxidized to the corresponding aldehyde. A Reformatsky reaction then took the route as far as **4.6**. The hydroxyl was silylated, and the ester was hydrolyzed. The resulting acid **4.8** was converted into its acid chloride, using oxalyl chloride, and condensation with amine **3.6** gave amide **5.1**


THPO
X
O
N
H
O
5.1 X = OSiMe $_2$Bu-*t*
Bu$_4$NF, 100%;
Swern
THPO
HO
O
N
O
5.2
TsOH, 56% overall
HO
O
N
O
5.3
Dess-Martin, 66%
O
O
N
O
5.4
HONH$_2$.HCl, AcONa, 62%
HO-N
O
N
O
2.1

Scheme 5

(Scheme 5).

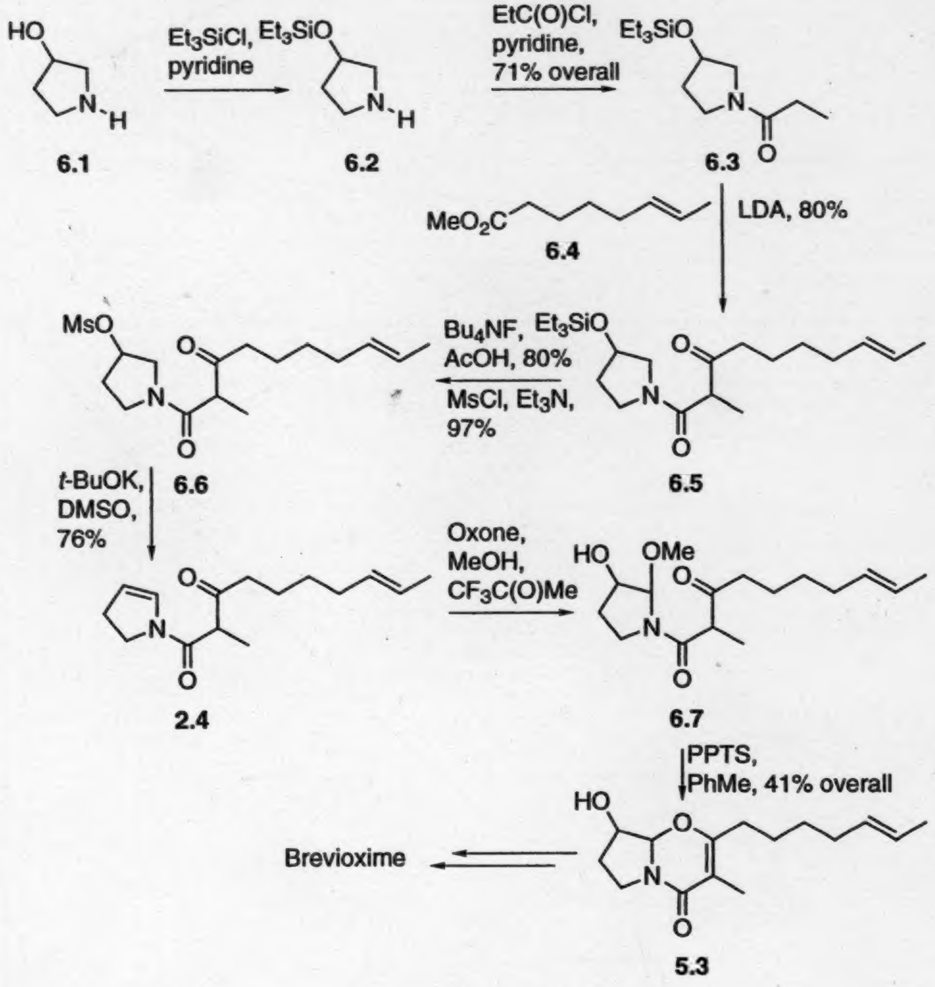
Selective deprotection and Swern oxidation afforded the hemiaminal **5.2**, which was in equilibrium with a trace amount of the corresponding ring-opened aldehyde. Treatment with TsOH effected cyclization and removal of the tetrahydropyranyl group, and alcohols **5.3** were obtained as a mixture of isomers (ca 1:1). Dess-Martin oxidation gave the expected ketone (**5.4**), and treatment with $HONH_2.HCl$ under standard conditions[10] produced a mixture (ca 1:1) of *Z*- and *E*-oximes. These isomerized during crystallization, and only the desired *E*-oxime was obtained.

Since the diastereoisomers of **5.3** were easily separable, use of optically pure malic acid should afford optically pure brevioxime (if there is no epimerization in the conversion of **5.1** into **5.2**), but this sequence does not appear to have been reported.

Clark's synthesis

Clark, working at DuPont, devised a synthesis of (±)-brevioxime, which was also adaptable to the synthesis of the natural (-)-antipode, although the compound was not obtained optically pure.[11]

3-Hydroxypyrrolidine was silylated and then acylated with propionyl chloride (Scheme 6, **6.1** → **6.2** → **6.3**). Amide **6.3** was then deprotonated and condensed with 0.5 equivalents of ester **6.4**, so as to form the β-keto amide **6.5**. Desilylation, mesylation, and treatment with *t*-BuOK served to


HO
6.1
Et_3SiCl, pyridine
Et_3SiO
6.2
EtC(O)Cl, pyridine, 71% overall
6.3
MeO_2C
6.4
LDA, 80%
6.5
Bu_4NF, AcOH, 80%
MsCl, Et_3N, 97%
MsO
6.6
t-BuOK, DMSO, 76%
2.4
Oxone, MeOH, $CF_3C(O)Me$
HO OMe
6.7
PPTS, PhMe, 41% overall
HO
5.3
Brevioxime

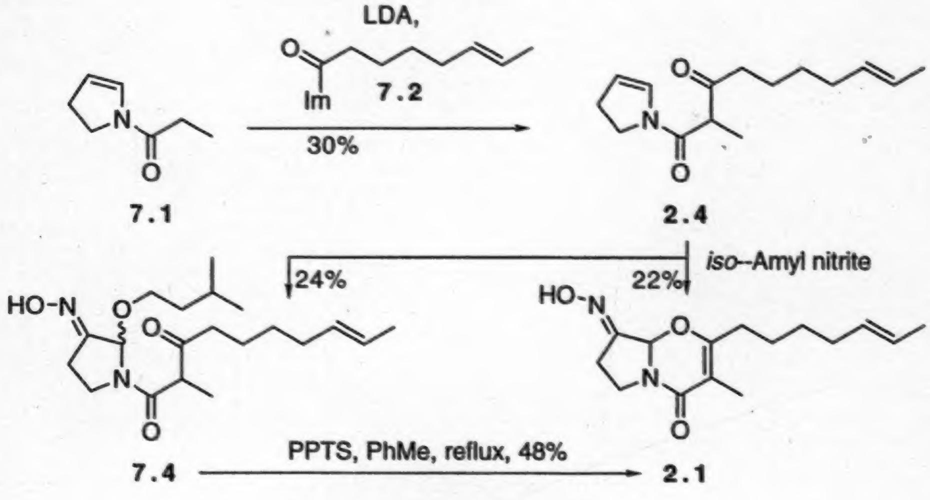
Scheme 6

generate the required double bond (**6.5** → **6.6** → **2.4**), each of the steps proceeding in good yield. Oxidation with Oxone in the presence of MeOH gave the hemiaminal methyl ethers **6.7**, and these were cyclized by heating with pyridinium *p*-toluenesulfonate (**6.7** → **5.3**). The resulting alcohols, which could be readily separated, were oxidized using the Dess-Martin reagent, and the resulting ketone was converted into its *E*-oxime [i.e. (±)-brevioxime].

In an effort to obtain optically active brevioxime, **2.4** was treated with (*S*,*S*)-(+)-*N*,*N*′-bis(3,3-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminomanganese(III) chloride to afford material corresponding to **6.7**. This was then processed as for the racemic series to give optically active brevioxime, but the compound was of low ee (probably 19%).

Parsons’synthesis

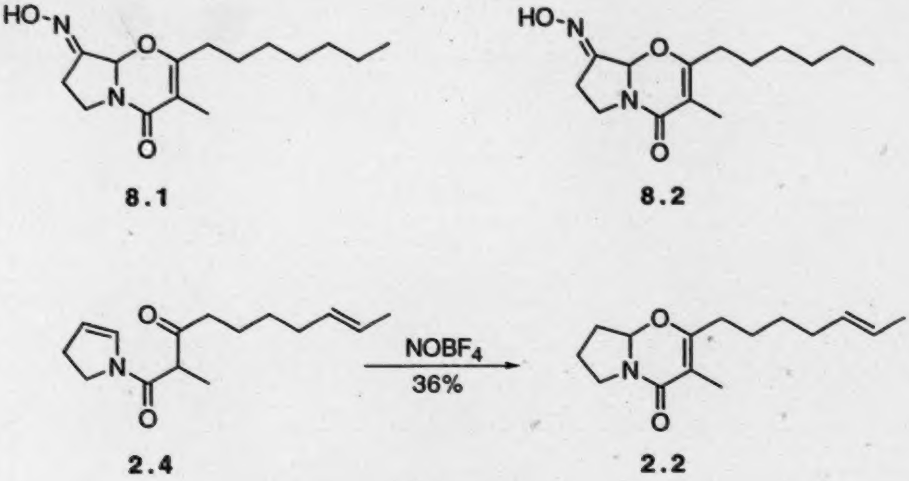
Quite recently, Karadogan and Parsons have described a short route (Scheme 7) to (±)-brevioxime.[12] It is based on the approaches of the prior syntheses, and its main innovative feature is to combine the final cyclization with formation of an oxime function.



Scheme 7

Enamide **7.1**, was prepared by the literature method (which will be described in the section dealing with our own synthesis), and the derived enolate was condensed with imidazolide **7.2**. This experiment afforded the coupled product **2.4** in 30% yield. Simultaneous cyclization and oximation occurred on treating **2.4** with *iso*-amyl nitrite. Two products were formed: brevioxime and the α-alkoxy oxime **7.4**. The latter was converted into brevioxime on heating in the presence of pyridinium *p*-toluenesulfonate.

The simple brevioxime analogs **8.1** and **8.2** were made similarly, and an attempt to use $NOBF_4$ as the nitrosating agent served to convert **2.4** into the natural product **2.2**, presumably by the action of adventitious HBF_4.

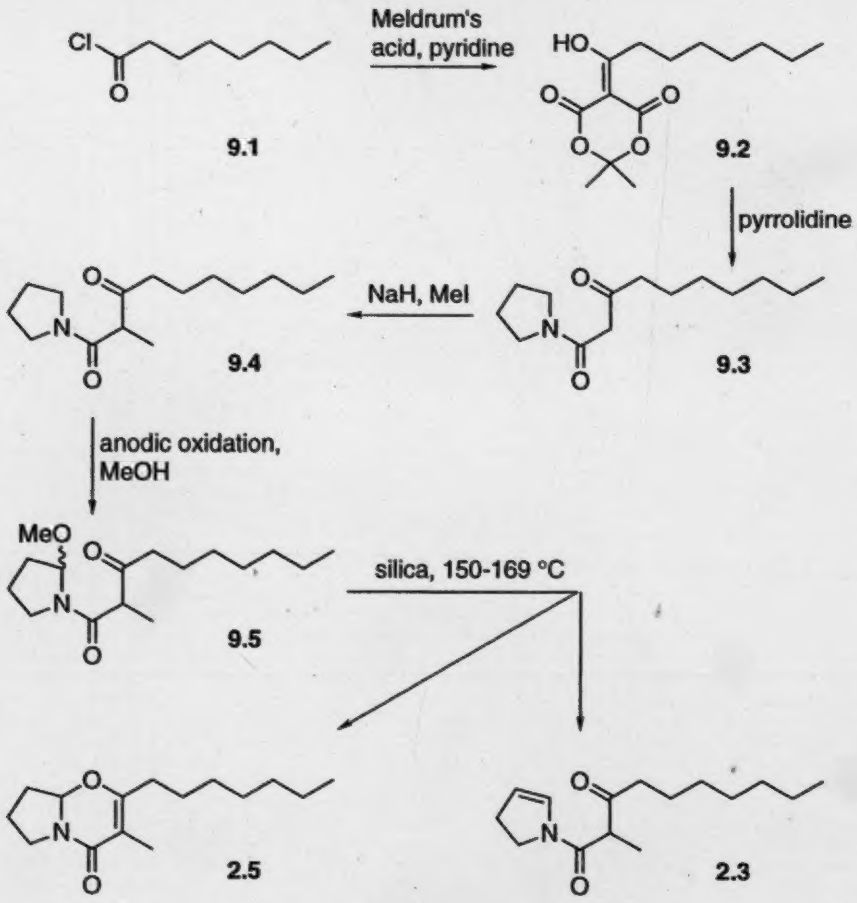


Scheme 8

Synthesis of related compounds

The group that isolated brevioxime also made the related compounds **2.3**, **2.5**, **2.2** and **2.4** – all except **2.5** being natural products isolated from *Penicillium brevicompactum*.[4,5]

Acylation of Meldrum's acid with octanoyl chloride gave the enolized triketone **9.2**, which, without purification, was


Meldrum's acid, pyridine
9.1
9.2
pyrrolidine
NaH, MeI
9.4
9.3
anodic oxidation, MeOH
9.5
silica, 150-169 °C
2.5
2.3

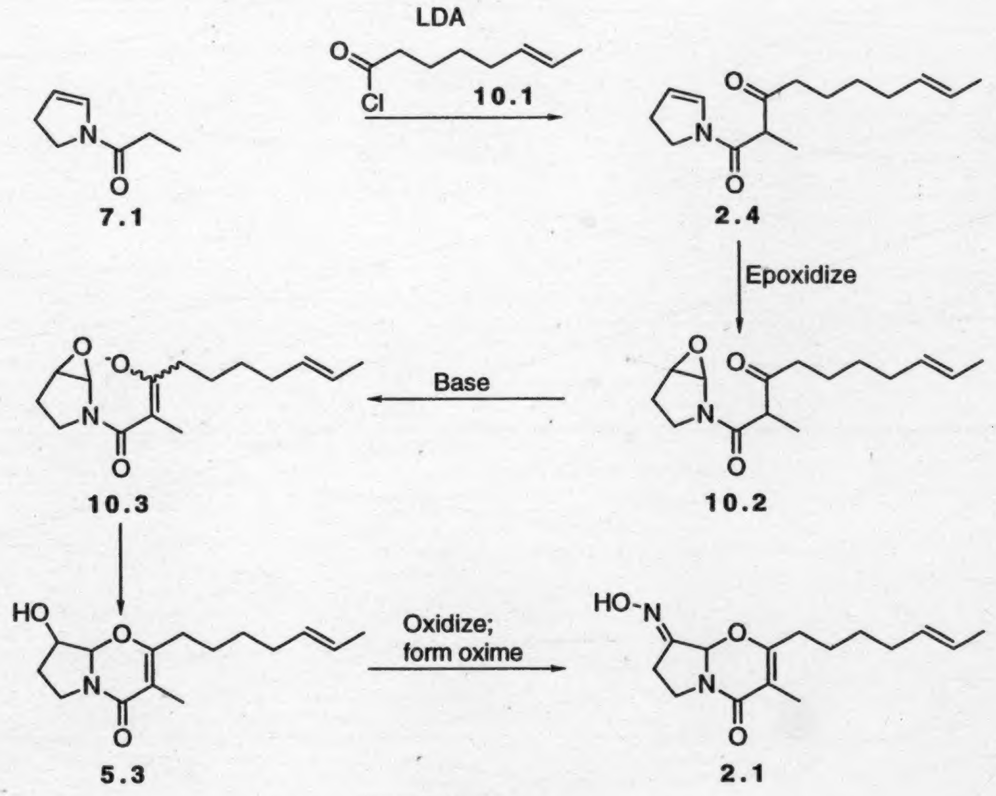
Scheme 9

heated with pyrrolidine, so as to form the β-keto amide **9.3**. Methylation in the usual way (NaH, MeI, DMF) and anodic oxidation generated the hemiaminal methyl ethers **9.5**. These were adsorbed on silica gel and the solid was heated at 150-160 °C. Under these conditions a separable 1:1 mixture of **2.5** and **2.3** was obtained. Compound **2.3** shows anti-juvenile hormone activity, while **9.3** and **9.4** showed strong knockdown toxicity to *Oncopeltus fasciatus*. The corresponding compounds with an olefinic side chain (**2.2** and **2.4**) were made by the same route but using 6-octenoyl chloride.[4]

DISCUSSION OF RESEARCH RESULTS

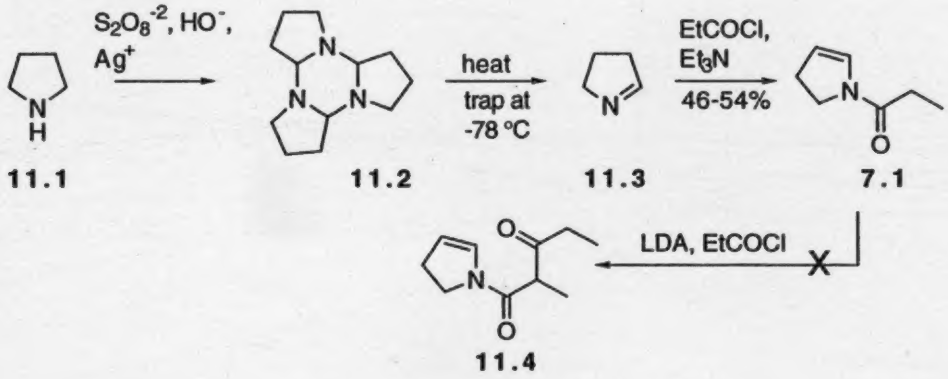
Synthesis of (±)-brevioxime

Our first approach is summarized in Scheme 10. Acylation of the enamide **7.1** with acid chloride **10.1** was expected to afford the required carbon skeleton of brevioxime (**7.1** → **2.4**).


LDA
10.1
7.1
2.4
Epoxidize
Base
10.3
10.2
5.3
Oxidize; form oxime
2.1

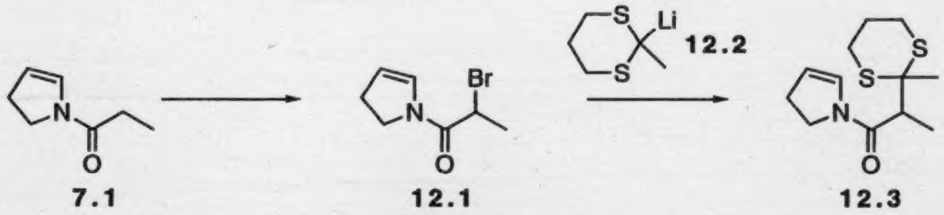
Scheme 10

We decided to test this approach by first making the β-keto enamide **11.4** (Scheme 11). To this end, we prepared enamide **7.1**, starting from pyrrolidine (**11.1**). The latter compound was oxidized under conditions that afforded the trimer **11.2**.[13] This was heated to afford **11.3**, and acylation with propionyl chloride[14] then gave **7.1** in 46-54% overall yield. We made one attempt to acylate the derived enolate (**7.1** → **11.4**). This was unsuccessful, and so we sought – perhaps prematurely – an alternative approach to compounds of type **11.4**.

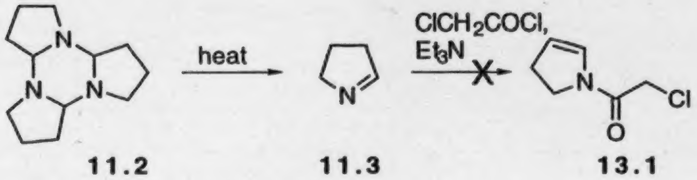

$S_2O_8^{-2}$, HO⁻, Ag⁺
heat
trap at -78 °C
EtCOCl, Et3N
46-54%
LDA, EtCOCl
11.1
11.2
11.3
7.1
11.4

Scheme 11

We decided to make **12.1** and then displace the halogen with a lithiated dithiane (Scheme 12). Treatment of **7.1** with LDA and NBS gave some of the desired bromo enamide **12.1**, but the yield was too low (8%) to be useful. The related compound **13.1** is known,[14] but our attempt to prepare it (Scheme 13) was unsuccessful.

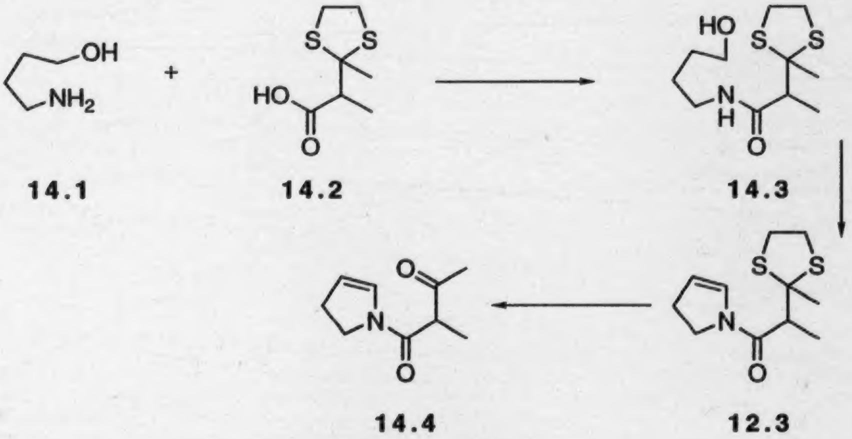

12.2
7.1
12.1
12.3

Scheme 12



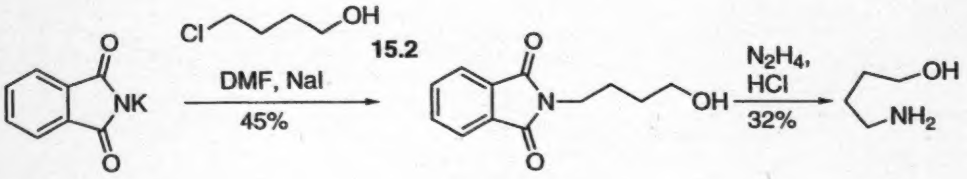
Scheme 13

Although these preliminary experiments had not been pursued with determination, we decided to modify our approach to brevioxime in such a way that a *preformed* 1-pyrroline unit was not used, but was generated *in situ* after the complete carbon skeleton had been assembled. The new plan is illustrated in Scheme 14, for preparation of the simple intermediate **14.4**.



Scheme 14

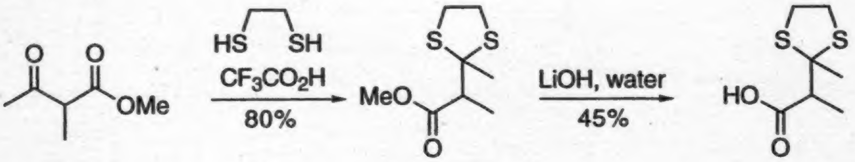
Amine **14.1**[15] was prepared by a standard Gabriel synthesis (Scheme 15) from 4-chlorobutanol (**15.2**). The yields were poor in each of the two steps, and we made no attempt to improve them.


Cl
OH
15.2
DMF, NaI
45%
NK
O
O
N
OH
N$_2$H$_4$,
HCl
32%
OH
NH$_2$

15.1 **15.3** **14.1**

Scheme 15

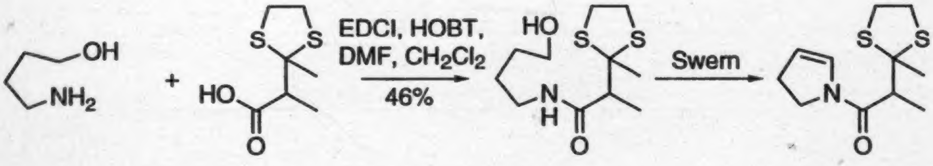
The other component (**14.2**) was also made in two steps (Scheme 16). Thioketalization of the β-keto ester **16.1**[16] worked smoothly when CF$_3$CO$_2$H was used as the catalyst[17] but an attempt to use Me$_3$SiCl[18] did not work. Hydrolysis with LiOH then gave the required acid (**14.2**).


O
O
OMe
HS
SH
CF$_3$CO$_2$H
80%
MeO
S
S
O
LiOH, water
45%
HO
S
S
O

16.1 **16.2** **14.2**

Scheme 16

Amine **14.1** and acid **14.2** were coupled using the standard EDCI method (Scheme 17); a single attempt at the coupling, using DCC and DMAP was unsuccessful. Swern oxidation of

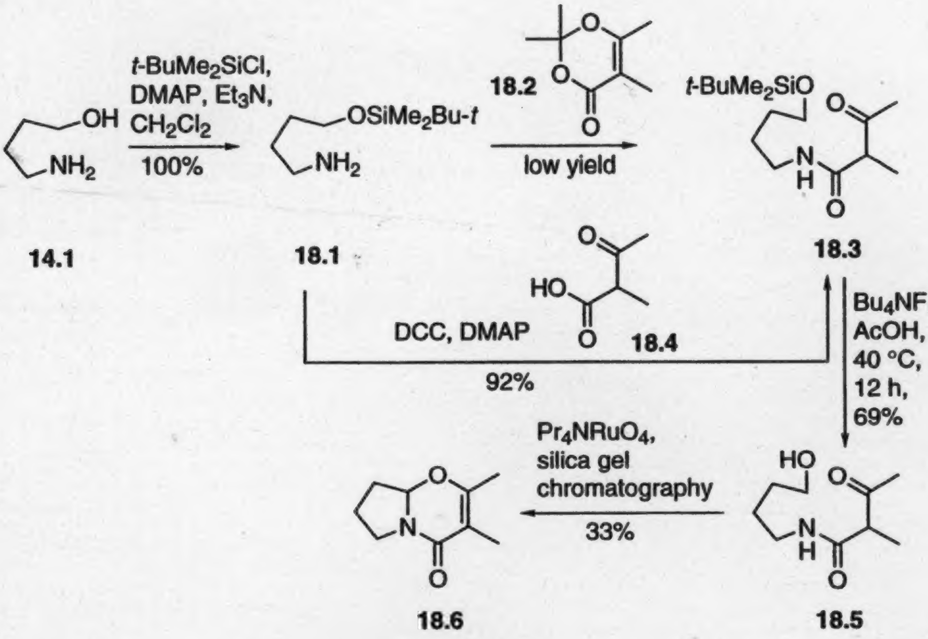

OH
NH$_2$
+
HO
S
S
O
EDCI, HOBT,
DMF, CH$_2$Cl$_2$
46%
HO
S
S
N
H
O
Swern
S
S
N
O

14.1 **14.2** **14.3** **12.3**

Scheme 17

alcohol **14.3** gave some of the desired *N*-acyl pyrroline **12.3**, but the yield was very low. Rather than attempt to improve it, we decided to deprotect the latent ketone group before oxidizing the primary alcohol. Unfortunately, the standard deprotection conditions we tried ($HgCl_2$, $CaCO_3$, MeCN, water[19] or $HgCl_2$, MeCN, water) gave none of the desired product, although the starting dithioketal was destroyed in both cases.

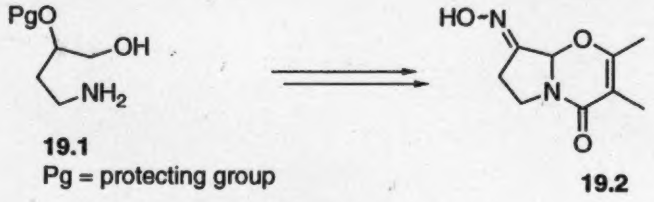
These observations suggested that the β-dicarbonyl unit should be installed in an unprotected form, and we decided, in view of the low yield we had obtained in an earlier coupling (see **14.1** + **14.2** → **14.3**), to use an amino alcohol in which the hydroxyl was protected. Thus, amino alcohol **14.1** was silylated (Scheme 18), and the resulting amine


OH
NH2
14.1
t-BuMe2SiCl,
DMAP, Et3N,
CH2Cl2
100%
OSiMe2Bu-t
NH2
18.1
18.2
O
O
O
low yield
t-BuMe2SiO
O
N
H
O
18.3
O
HO
O
18.4
DCC, DMAP
92%
Bu4NF,
AcOH,
40 °C,
12 h,
69%
Pr4NRuO4,
silica gel
chromatography
33%
O
N
O
18.6
HO
O
N
H
O
18.5

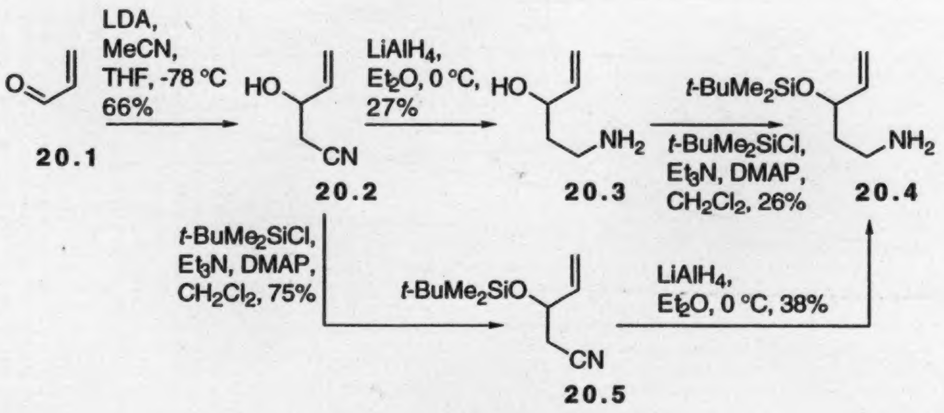
Scheme 18

(**18.1**[20]) was condensed efficiently with β-keto acid **18.4**,[21] using DCC. The keto acid is sensitive, and so we also tried to effect coupling with the dioxinone **18.2**.[22] Some of the desired coupled product was indeed formed, but the yield was low, and we had to accept the slight inconvenience of working with keto acid **18.4**.

Desilylation of **18.3** and TPAP oxidation allowed us to isolate the desired model **18.6** in 33% yield. Appropriate ^1H NMR measurements showed that the intermediate aldehyde cyclizes while in contact with silica gel.[23] CF_3CO_2H and TsOH can also induce cyclization, but the yield was no higher than with silica gel. The formation of **18.6** seemed to validate the approach based on amide closure onto an aldehyde group,


PgO
OH
NH2
19.1
Pg = protecting group
HO-N
O
N
O
19.2

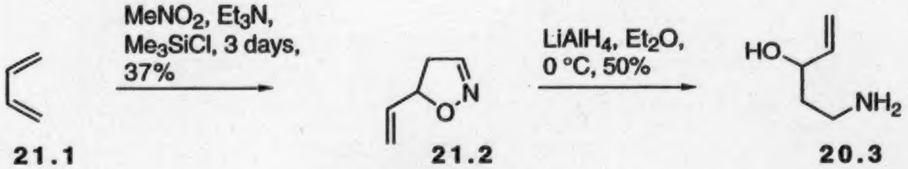
Scheme 19


O
20.1
LDA,
MeCN,
THF, -78 °C
66%
HO
CN
20.2
LiAlH4,
Et2O, 0 °C,
27%
HO
NH2
20.3
t-BuMe2SiO
t-BuMe2SiCl,
Et3N, DMAP,
CH2Cl2, 26%
NH2
20.4
t-BuMe2SiCl,
Et3N, DMAP,
CH2Cl2, 75%
t-BuMe2SiO
CN
20.5
LiAlH4,
Et2O, 0 °C, 38%

Scheme 20

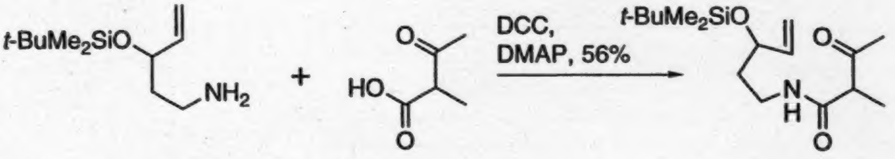
followed by another cyclization involving the β-ketone function, and we next sought to prepare the more advanced model **19.2**, by an analogous route, so as to gain experience in generating the oxime function. For this purpose, the required amino unit was of type **19.1**, and our initial approach to a precursor of this class is shown in Scheme 20.

Our plan was to make amino olefin **20.4**, and to then cleave the double bond after acylation of the amino group with β-keto acid **18.4**. The amino olefin was prepared as follows: Deprotonation of MeCN and condensation with acrolein gave the hydroxy nitrile **20.2**,[24] but $LiAlH_4$ reduction afforded the amine **20.3**[25] in only 27% yield. This was silylated, to produce the *O*-protected amine **20.4**.[26] Reversing the order of the last two steps improved the overall yield, but only to 29% (Scheme 20). We also looked briefly at a different approach (Scheme 21). Butadiene was converted (37%) into the isoxazoline **21.2** by the literature procedure,[27] and $LiAlH_4$ reduction then gave alcohol **20.3**.[27] The route summarized in Scheme 21 was not pursued, as a better route (see later) was developed at about the same time.



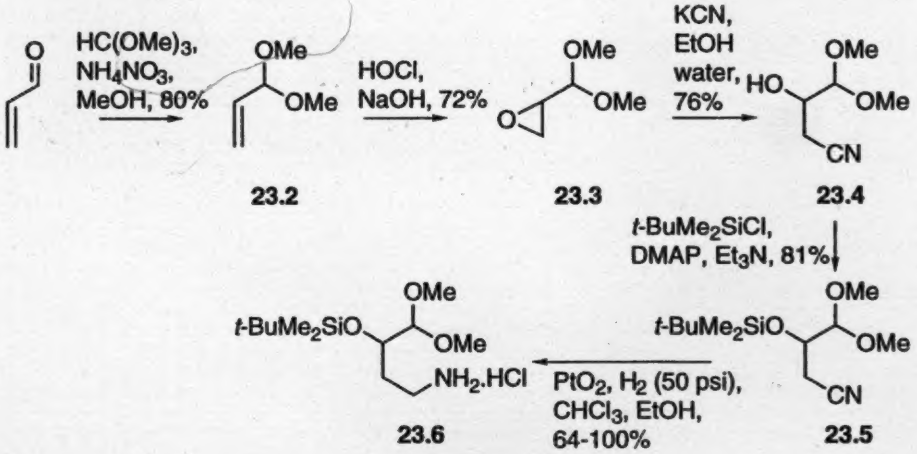
Scheme 21

The reduction of our aliphatic nitriles by $LiAlH_4$ does not appear to be an easy process, and we eventually found a route that avoids this troublesome step. However, with some of the protected amine **20.4** in hand, we proceeded to couple the material with acid **18.4**, using DCC as the coupling reagent (Scheme 22). When we attempted to cleave the double bond in the product (**22.1**) with O_3 (Ph_3P reduction) we were unable to isolate characterizable products. We did not try OsO_4-$NaIO_4$ but, instead, prepared compound **23.6** in which the aldehyde function was protected as a dimethyl acetal rather than as a double bond. In the event, this approach proved to


t-BuMe2SiO
NH2
+
HO
O
O
DCC,
DMAP, 56%
t-BuMe2SiO
N
H
O
O

20.4 **18.4** **22.1**

Scheme 22

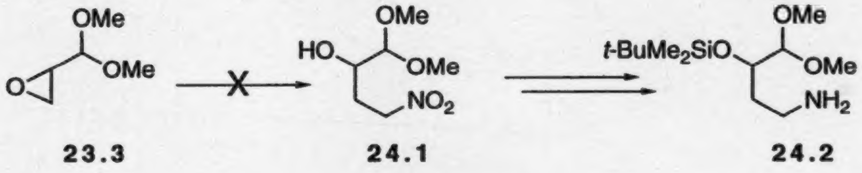

O
HC(OMe)3,
NH4NO3,
MeOH, 80%
OMe
OMe
23.2
HOCl,
NaOH, 72%
O
OMe
OMe
23.3
KCN,
EtOH
water,
76%
HO
OMe
OMe
CN
23.4
t-BuMe2SiCl,
DMAP, Et3N, 81%
t-BuMe2SiO
OMe
OMe
CN
23.5
PtO2, H2 (50 psi),
CHCl3, EtOH,
64-100%
t-BuMe2SiO
OMe
OMe
NH2.HCl
23.6

Scheme 23

be successful and was used in the synthesis of brevioxime.

Acrolein was converted into its dimethyl acetal[28] and this was epoxidized with NaOCl, following a standard general[29] procedure. Epoxide opening with KCN in aqueous EtOH[30] proceeded without incident, and the hydroxyl was protected by silylation under standard conditions (**23.4** → **23.5**). When we came to reduce the nitrile function we found the reaction very troublesome. $LiAlH_4$ did not give the required product, but we eventually found that catalytic hydrogenation over PtO_2 in the presence of several equivalents of $CHCl_3$,[31] which serves as a controlled source of HCl, gave the desired amine as its hydrochloride salt in yields varying from 64 to 100% yield. We did not attempt to identify the cause of the yield variation, but we did notice that the spent catalyst in the case of the quantitative reaction was inflammable. This method of reducing nitriles deserves to be better known.

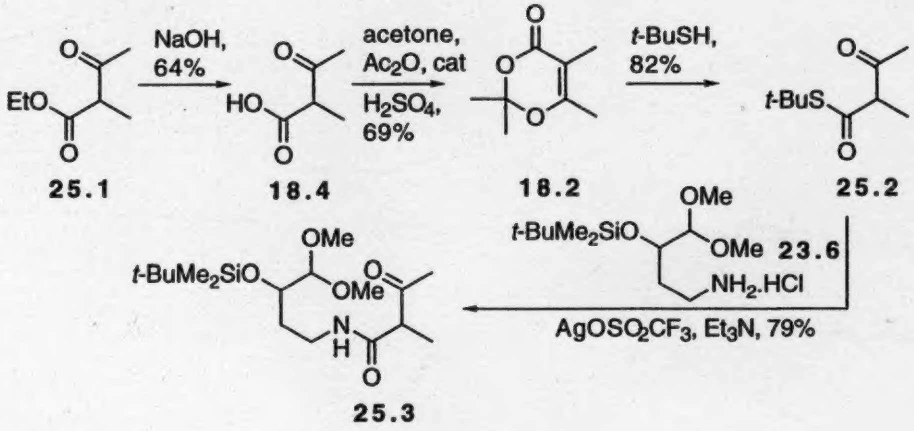
Our problems with the reduction had also caused us to look at the possibility of using a nitro compound (Scheme 24), as it was known in this laboratory[32] that the reduction of aliphatic nitro groups with $NaBH_4$-$NiCl_2.6H_2O$[33] works very well. However, the single attempt we made to prepare **24.1**



Scheme 24

($MeNO_2$, *t*-BuOK)[34] was unsuccessful, and we had by that time found the PtO_2-H_2-$CHCl_3$ method.

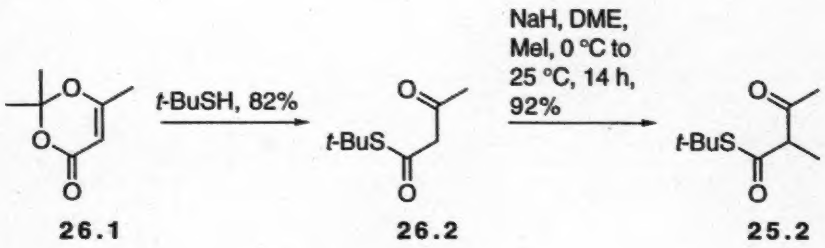
When amine **23.6** was coupled with keto acid **18.4**, using DCC, the required product was indeed formed but it was contaminated by significant amounts of *N,N'*-dicyclohexylurea, and purification was difficult. We turned, therefore, to the general method for the preparation of β-keto amides reported by Ley *et al.*[35] This involves Ag^+-mediated reaction of an amine with a β-keto thioester. The requisite material for the present case is thioester **25.2**. Hydrolysis of ester


NaOH,
64%
acetone,
Ac_2O, cat
H_2SO_4,
69%
t-BuSH,
82%
EtO
HO
t-BuS
O
25.1
18.4
18.2
25.2
OMe
t-BuMe$_2$SiO
OMe
NH_2.HCl
23.6
$AgOSO_2CF_3$, Et_3N, 79%
N
H
25.3

Scheme 25

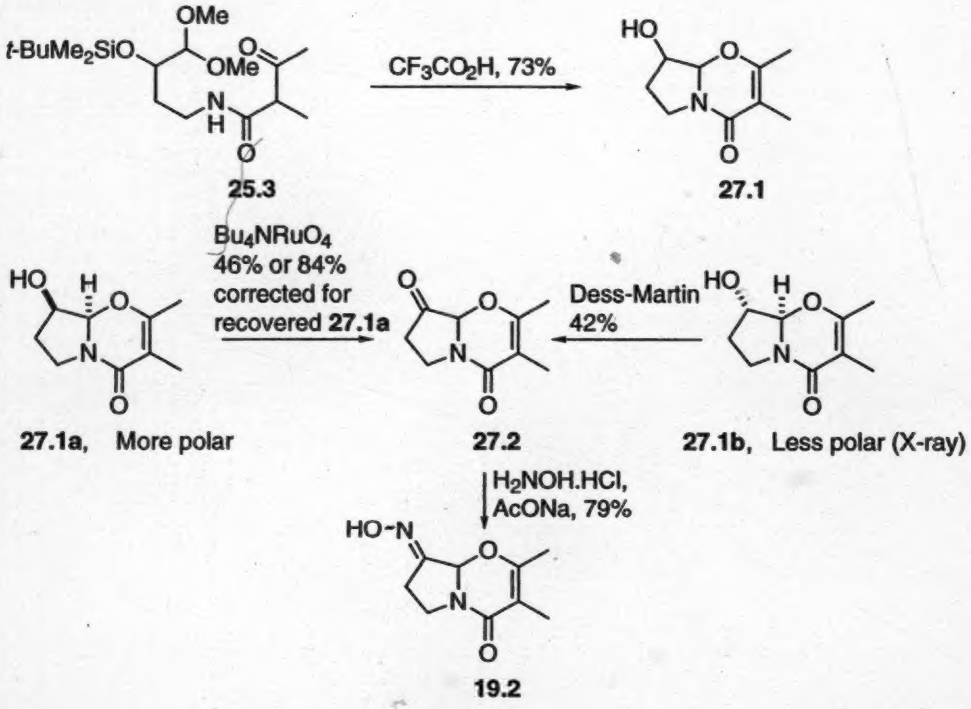
25.1[21] afforded the sensitive acid **18.4**, and this was converted into the dioxinone **18.2**, which reacted with *t*-BuSH, giving the desired β-keto thioester **25.2**.[36] Coupling of the thioester with the amine component **23.6** was achieved in 79% yield by using $AgOSO_2CF_3$ (**25.2** → **25.3**).

At this point a slight improvement in the preparation of thioester **25.2** was made. Acid **18.4** is unstable and handling this compound was now avoided by using[36] commercial **26.1** and alkylating[37] the derived β-keto thioester **26.2**, as summarized in Scheme 26.


t-BuSH, 82%
NaH, DME,
MeI, 0 °C to
25 °C, 14 h,
92%
t-BuS
t-BuS
26.1
26.2
25.2

Scheme 26

With **25.3** in hand, a number of conditions were examined to effect conversion into the desired bicyclic system ($BF_3.OEt_2$; Me_3SiCl, NaI, MeCN; oxalic acid, silica gel;


t-BuMe2SiO
OMe
OMe
CF3CO2H, 73%
HO
25.3
27.1
Bu4NRuO4
46% or 84%
corrected for
recovered 27.1a
Dess-Martin
42%
HO
HO
27.1a, More polar
27.2
27.1b, Less polar (X-ray)
H2NOH.HCl,
AcONa, 79%
HO-N
19.2

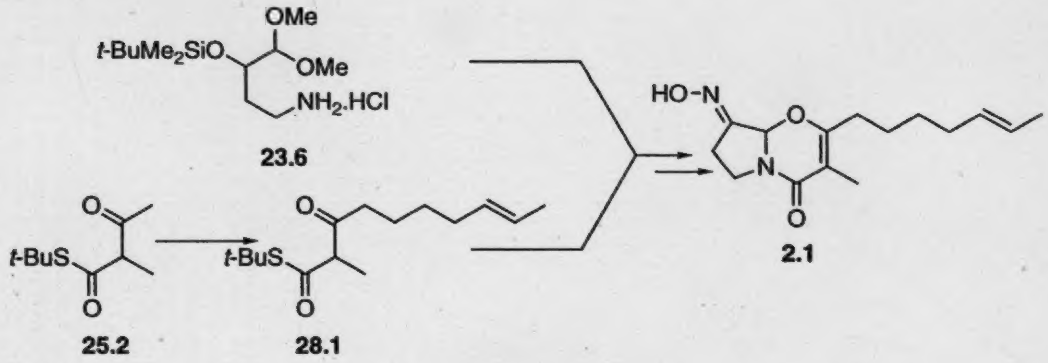
Scheme 27

$TsOH.H_2O$, acetone, reflux; CF_3CO_2H, $CHCl_3$), but only the use of CF_3CO_2H in $CHCl_3$ was successful and afforded **27.1** as a mixture of two isomers, the more polar material being obtained in 36% yield and the less polar in 37% yield. X-ray crystallographic analysis of the latter established that the hydroxyl and adjacent angular hydrogen are *syn* (see Appendix for X-ray data).

We tried to oxidize the more polar compound with the Dess-Martin reagent, but the substance was inert; use of Pr_4NRuO_4-NMO,[38] however, gave ketone **27.2** in 46% yield, or 84% after correction for recovered starting material. The less polar isomer was destroyed by Pr_4NRuO_4, but gave **27.2** in 42% yield with the Dess-Martin reagent. We interpret the different behavior of the isomers towards the two reagents in the following way. The bulky Dess-Martin reagent can approach only the *exo* hydroxyl, as in the less polar isomer. If the ruthenium reagent acts as a hydride acceptor[39] then it would react only with that isomer having an *exo* hydrogen on the carbon bearing the hydroxyl, as in the more polar isomer.

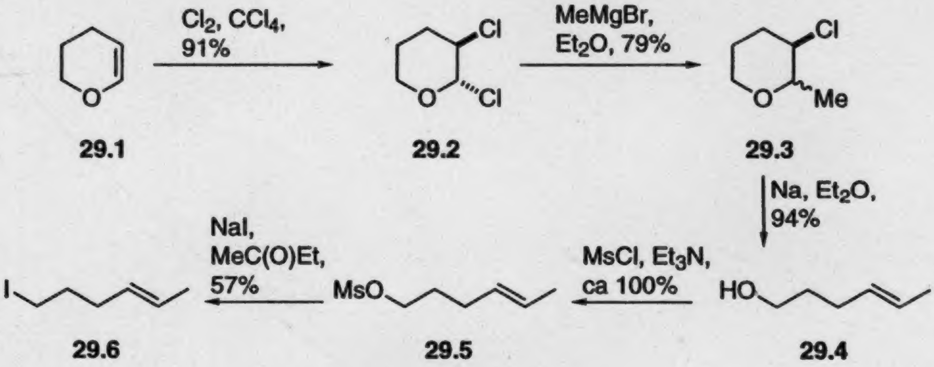
Treatment of ketone **27.2** with hydroxylamine under classical conditions[10] afforded in 73% yield the *E*-oxime together with a small amount (6% yield) of the *Z*-isomer.

With these encouraging results in hand, we embarked on the synthesis of brevioxime itself. For this purpose – by analogy with our model studies – we needed the two components **23.6** and **28.1** (Scheme 28), and our first task was to prepare the thioester **28.1**. Since ester **25.2** was available from our


OMe
t-BuMe2SiO
OMe
NH2.HCl
23.6
HO-N
O
N
O
2.1
O
t-BuS
O
25.2
O
t-BuS
O
28.1

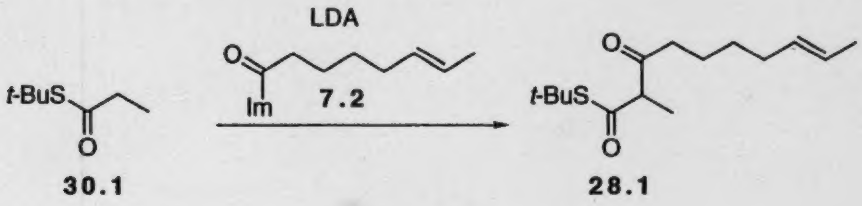
Scheme 28

model studies we naturally tried to alkylate it with (*E*)-6-iodo-2-hexene (**29.6**). This known iodide[40] was made by the classical route shown in Scheme 29,[41] the literature procedures for conversion of dihydropyran to alcohol **29.4** being easily repeatable. In the preparation of 3-chlorotetrahydro-2-methylpyran by the literature procedure,[41b] use of a mechanical stirrer is essential because, if the mixture is not stirred vigorously, the reaction becomes violent after 2/3 of the Grignard reagent has been added. The initial chlorination of dihydropyran was done in CCl_4 instead of Et_2O.


O
29.1
Cl2, CCl4,
91%
Cl
O
Cl
29.2
MeMgBr,
Et2O, 79%
Cl
O
Me
29.3
Na, Et2O,
94%
HO
29.4
MsCl, Et3N,
ca 100%
MsO
29.5
NaI,
MeC(O)Et,
57%
I
29.6

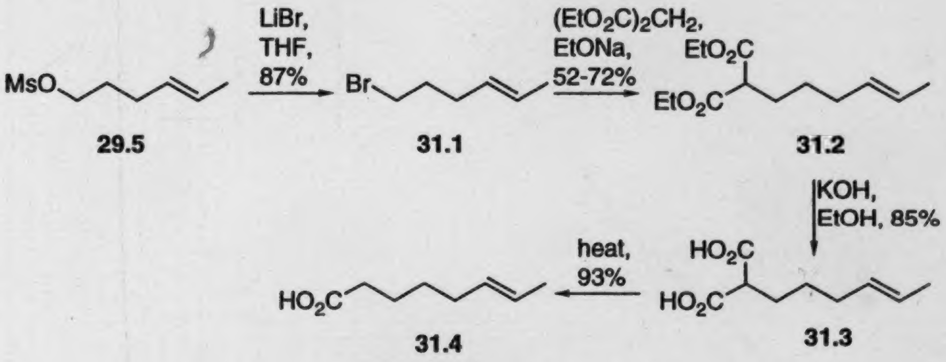
Scheme 29

Alcohol **29.4** is reported[41b] to contain <5% of the *Z*-isomer, and so the material was subjected to spinning band distillation to afford the pure *E*-olefin (20% recovery). The alcohol was converted into its mesylate, and displacement with NaI gave the required iodide **29.6**. When the iodide was used to alkylate the dianion[42] derived from **25.2**, we were disappointed to find that the yield of the desired **28.1** was very poor (33%). Almost the same result was obtained in alkylation of **26.2**. The inefficiency of these procedures caused us to adopt the route shown in Scheme 30.[43]


LDA
O
Im
7.2
t-BuS
O
30.1
O
t-BuS
O
28.1

Scheme 30

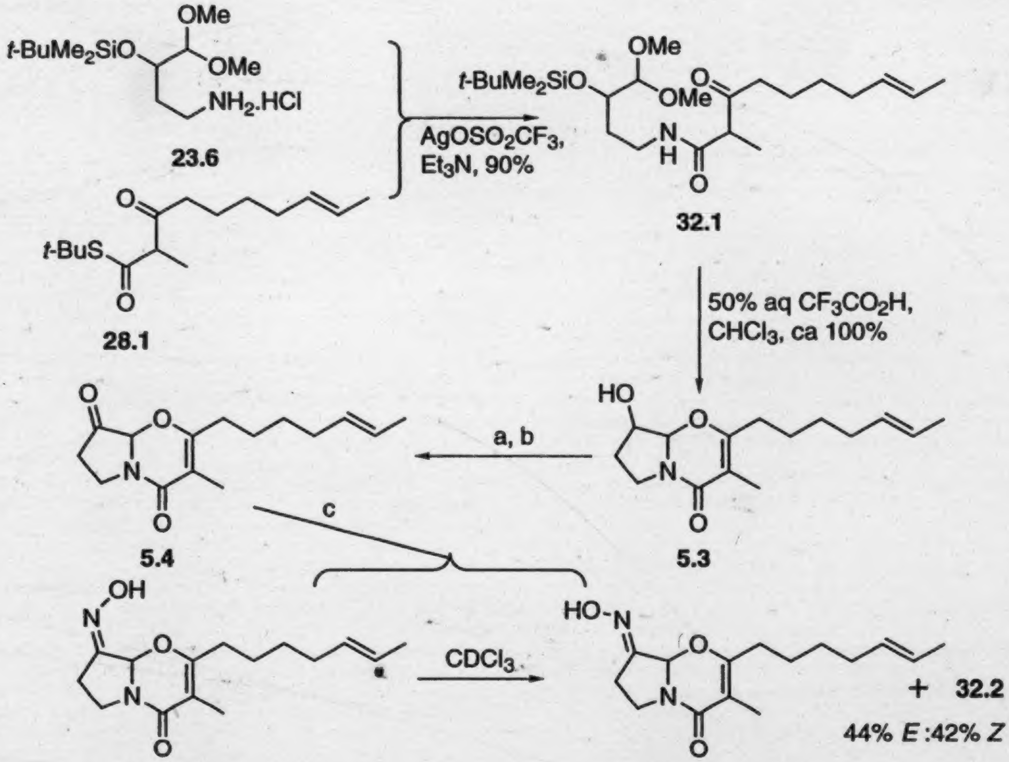
The imidazolide **7.2** was prepared (Scheme 31) from mesylate **29.5**. Mesylate **29.5** was converted into bromide


MsO
29.5
LiBr,
THF,
87%
Br
31.1
(EtO2C)2CH2,
EtONa,
52-72%
EtO2C
EtO2C
31.2
KOH,
EtOH, 85%
HO2C
HO2C
31.3
heat,
93%
HO2C
31.4

Scheme 31

31.1,[44] and simple displacement with malonate (**31.1** → **31.2**[45,46]), followed by base hydrolysis[46] and thermal decarboxylation[46] gave the required acid **31.4**. Although each of these steps had been reported in the literature, it is likely that the material described contained some of the *Z*-isomer.

The acid was converted into its imidazolide[43,47] by treatment with $Im_2C(O)$ and, without isolation, the imidazolide was added (Scheme 30) to the enolate (3 equiv.[43])


t-BuMe₂SiO
OMe
OMe
NH₂.HCl
23.6
t-BuS
28.1
AgOSO₂CF₃, Et₃N, 90%
32.1
50% aq CF₃CO₂H, CHCl₃, ca 100%
HO
5.3
a, b
5.4
c
32.2
CDCl₃
2.1
+ 32.2
44% E :42% Z

[a]Less polar isomer: Dess-Martin, 54% or 99% corrected for recovery of **5.3**.
[b]More polar isomer: Pr_4NRuO_4, 18% or 23% corrected for recovered **5.3**.
[c]$H_2NOH.HCl$, AcONa, 81% *E* isomer, 13% *Z* isomer

Scheme 32

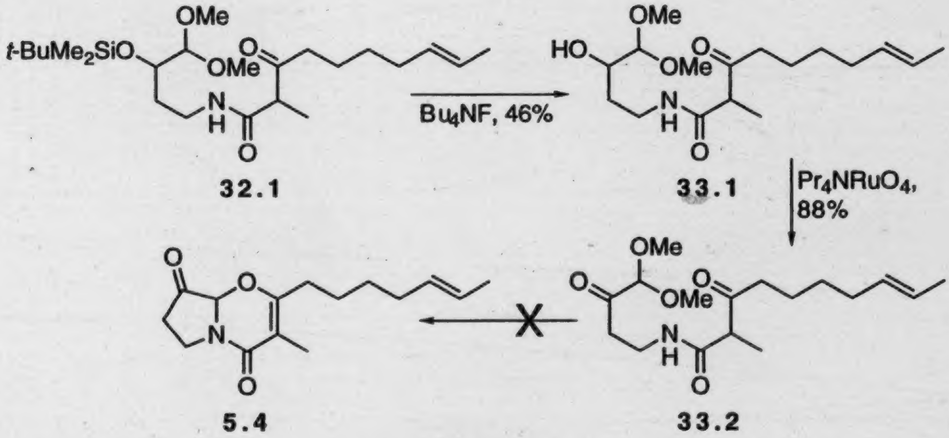
prepared from thioester **30.1**[49] by treatment with LDA. In this way, β-keto thioester **28.1** was obtained in 64% yield (Scheme 30).

Coupling of **28.1** with the amino component **23.6** under conditions that had served well in the model study ($AgOSO_2CF_3$, Et_3N) was again successful (Scheme 32) and gave the β-keto amide **32.1** in 90% yield. Exposure to a mixture of 50% aqueous CF_3CO_2H and $CHCl_3$ then resulted in the desired series of transformations (Scheme 32, hydrolysis, cyclization, and desilylation), giving rise to **5.3** as a 1:1 mixture of epimers in almost quantitative yield.

As in the model series, the next step – oxidation of the hydroxyl to a ketone – was not straightforward. The less polar alcohol gave ketone **5.4** in 54% yield (or 99%, corrected for recovered starting material) with the Dess-Martin reagent; Pr_4NRuO_4 destroyed the alcohol. The more polar alcohol could be oxidized by Pr_4NRuO_4 in 18% yield (or 23%, after correction for recovered starting material). This alcohol was inert to the Dess-Martin reagent. Thus, the total yield of ketone **5.4** is about 39%. Comparison of the ^1H NMR spectra of the two alcohols with those of the model compounds **27.1b** and **27.1a** suggests[50] that once again, the less polar isomer has the hydroxyl and angular hydrogen *syn*.

Ketone **5.4** was converted into a 4.3:1 (^1H NMR) mixture of separable oximes. The major isomer (81% yield) proved to have the natural *E*-geometry (**2.1**). When the minor isomer **32.2** (13% yield) was stored for 2 days in $CDCl_3$, it was converted into a mixture of the *E*- and *Z*-oximes, which were isolated in yields of 44% and 42%, respectively. Our racemic brevioxime was crystalline and its spectral properties were identical (within experimental error) with reported[2] values.

We tried to bypass the above difficulties in the oxidation step by performing the oxidation prior to the assembly of the bicyclic ring system. Desilylation of **32.1** gave alcohol **33.1** in 46% yield (Scheme 33). We deferred improvement of this yield until we had examined the subsequent steps. Alcohol **33.1** was oxidized to the corresponding ketone **33.2** but, unfortunately, we were unable to effect the hydrolysis-cyclization sequence under conditions that had worked previously or in the presence of $TsOH.H_2O$, or Me_3SiCl-NaI,[51] $SnCl_2$,[52] DDQ,[53] or Amberlyst 115.[54] Consequently, we decided to accept the synthesis we had already completed.



Scheme 33

Conclusion

The above work illustrates the utility of the cyclization of an amide nitrogen onto an aldehyde carbonyl for generating certain nitrogen heterocycles. The method we have used to make brevioxime is convergent, and is clearly suitable for the synthesis of analogs.

As described in the review section, another synthesis very similar to ours was completed before our own work, but was not published until long after we had reported our results. In our studies we prepared two simplified models for brevioxime, and those experiments establish that our approach can be used to make analogs. Clearly the length of the C_7 substituent can be varied, as well as its constitution. It should also be possible to prepare *O*-alkyl derivatives of the oxime function, but we do not know if separation of *Z* and *E* isomers would then be a simple matter.

EXPERIMENTAL SECTION

General Procedures. Unless stated to the contrary, the following conditions apply: Reactions were carried out under a slight static pressure of N_2 that had been purified by passage through a column (3.5 x 42 cm) of R-311 catalyst[55] and then through a similar column of Drierite. Glassware was dried in an oven for at least 3 h before use (120 °C) and either cooled in a desiccator over Drierite, or assembled quickly, sealed with rubber septa, and allowed to cool under a slight static pressure of N_2. Reaction mixtures were stirred by Teflon-coated magnetic stirring bars.

Hexane used for chromatography was distilled before use.

Products were isolated from solution by evaporation under water-aspirator vacuum at, or below, room temperature, using a rotary evaporator.

Cannula transfers were done under slight pressure (N_2), not by suction.

Commercial thin layer chromatography (TLC) plates (silica gel, Merck 60F-254) were used. Spots were detected by spraying the plate with a solution of phosphomolybdic acid[56] or *p*-anisaldehyde,[57] followed by charring with a heat gun, or by examination under UV light. Silica gel for flash chromatography was Merck type 60 (230-400 mesh).

Dry solvents were prepared under an inert atmosphere and transferred by syringe or cannula. Dry THF and Et_2O were distilled from sodium and benzophenone ketyl.

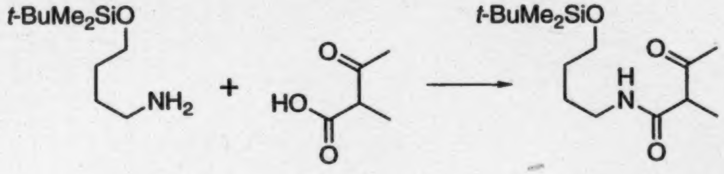
FT-IR measurements were recorded on a Nicolet 7000 FTIR instrument. Measurements were made as casts from the specified solvent using potassium bromide plates.

^1H nuclear magnetic resonance spectra were recorded with Bruker AM-300 (at 300 MHz), Varian INOVA-300 (at 300 MHz), Bruker AM-360 (at 360 MHz) or Bruker AM-400 (at 400 MHz) spectrometers in the specified deuterated solvent at 27.2 °C. ^{13}C spectra were recorded with Bruker AM-300 (at 75.5 MHz) or Varian UNITY-500 (at 125 MHz) at 27.2 °C. The symbols s', d', t', and q' used for ^{13}C NMR signals indicate 0, 1, 2, or 3 attached hydrogens, respectively, which are assigned based on the APT experiment.

Mass spectra were recorded with AEI Models MS-12, MS-50 MS9 (modified), Kratos MS50 (modified) or Micromass ZabSpec Hybrid Sector-TOF mass spectrometers. For isotope peaks, high-resolution mass data were taken from the highest mass number peak shown in the spectrum.

Compounds isolated by flash chromatography were pure by TLC and, unless otherwise stated, also as judged by high field ^1H and ^{13}C NMR spectra.

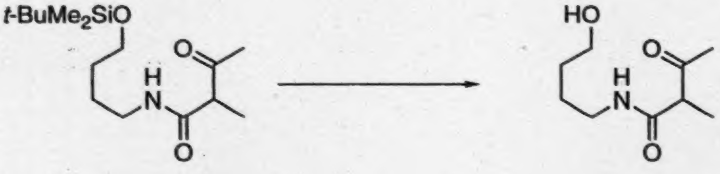
N-[4-[[Dimethyl(1,1-dimethylethyl)silyl]oxy]-butyl]-2-methyl-3-oxobutanamide (18.3).



18.1 **18.4** **18.3**

A cold (-78 °C) solution of β-keto acid **18.4**[21a] (310 mg, 2.66 mmol) in dry CH_2Cl_2 (0.50 mL) was added dropwise (ca 5 min) to a stirred and cooled (-78 °C) solution of the *O*-protected amino alcohol **18.1**[20] (300 mg, 1.48 mmol), DCC (335 mg, 1.62 mmol) and DMAP (30 mg, 0.24 mmol) in dry CH_2Cl_2 (1 mL). Stirring was continued overnight, but the cold bath was not recharged. The mixture was filtered and evaporated. Flash chromatography of the residue over silica gel (2 x 22 cm), using 1:1 EtOAc-hexanes, gave **18.3** (411 mg, 92%) as a colorless oil: FTIR ($CDCl_3$ cast) 3500-3150, 1724, 1641 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.04 (s, 6 H), 0.88 (s, 9 H), 1.37 (d, *J* = 7.0 Hz, 3 H), 1.47-1.60 (m, 4 H), 2.23 (s, 3 H), 3.20-3.30 (m, 2 H), 3.36 (q, *J* = 7.2 Hz, 1 H), 3.62 (t, *J* = 5.9 Hz, 2 H), 6.28 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ -5.3 (q′), 14.7 (q′), 18.4 (s′), 26.0 (q′), 26.1 (t′), 28.6 (q′), 30.0 (t′), 39.5 (d′), 55.1 (t′), 62.7 (t′), 169.3 (s′), 207.6 (s′); exact mass *m/z* calcd for $C_{15}H_{31}NO_3Si$ 301.2073, found 301.2074. This experiment was done several times; the yields varied between 67% and 92%.

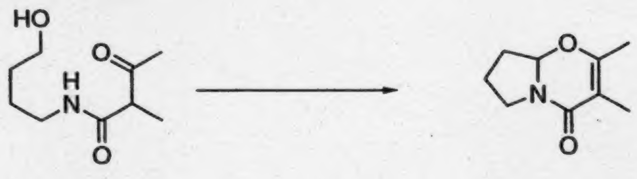
***N*-(4-Hydroxybutyl)-2-methyl-3-oxobutanamide (18.5).**



18.3 **18.5**

Bu_4NF (1.0 M in THF, 0.81 mL, 0.81 mmol) was added to a stirred solution of β-keto amide **18.3** (222 mg, 0.74 mmol) and glacial AcOH (0.09 mL, 1.47 mmol) in dry THF (2.50 mL). The mixture was warmed to 45 °C (oil bath) for 14 h, and then evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 1:9 MeOH-Et_2O, gave **18.5** (110 mg, 80%) as a colorless oil: FTIR ($CDCl_3$ cast) 3650-3150, 1720, 1647 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.37 (d, *J* = 7.3 Hz, 3 H), 1.54-1.66 (m, 4 H), 2.24 (s, 3 H), 2.20-2.28 (br s, 1 H), 3.22-3.32 (m, 2 H), 3.38 (q, *J* = 7.2 Hz, 1 H), 3.66 (t, *J* = 5.8 Hz, 2 H), 6.54 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 14.7 (q′), 26.1 (t′), 28.6 (q′), 29.7 (t′), 39.4 (t′), 55.0 (d′), 62.3 (t′), 169.6 (s′), 207.7 (s′); exact mass *m/z* calcd for $C_9H_{17}NO_3$ 187.1209, found 187.1212.

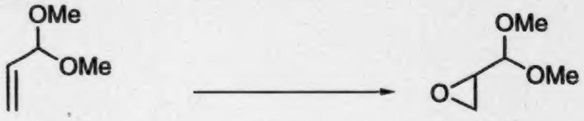
6,7,8,8a-Tetrahydro-2,3-dimethyl-4*H*-pyrrolo[2,1-b][1,3]oxazine-4-one (18.6).



18.5 **18.6**

N-Methylmorpholine *N*-oxide (24.0 mg, 0.20 mmol), Pr_4NRuO_4 (2.4 mg, 0.007 mmol) and crushed 4Å molecular sieves (68 mg) were added to a stirred solution of alcohol **18.5** (25.5 mg, 0.14 mmol) in dry CH_2Cl_2 (0.8 mL). Stirring was continued for 2 h, and the mixture was then loaded onto a silica gel column (0.8 x 14 cm). Flash chromatography, using 99:1 EtOAc-hexanes, gave **18.6** (8.2 mg, 33%) as an unstable, colorless oil: FTIR ($CDCl_3$ cast) 1655 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.80 (s, 3 H), 1.81-1.92 (m, 1 H), 1.93 (s, 3 H), 1.94-2.16 (m, 2 H), 2.22-2.35 (m, 1 H), 3.38-3.48 (m, 1 H), 3.70-3.78 (m, 1 H), 5.24 (t, *J* = 5.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 10.3 (q′), 16.7 (q′), 21.9 (t′), 31.6 (t′), 44.3 (t′), 87.4 (d′), 106.6 (s′), 160.2 (s′); exact mass *m/z* calcd for $C_9H_{13}NO_2$ 167.0946, found 167.0946.

1,1-Dimethoxy-2,3-epoxypropane (23.3).

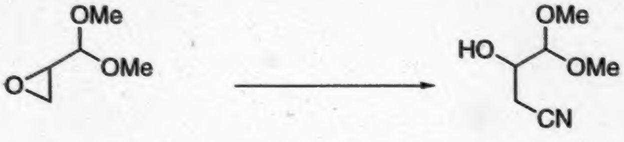


23.2 **23.3**

The method[29] for the corresponding diethyl acetal was followed. Ice-cold HOCl[58] (79.0 mL) was added in three portions to a stirred and cooled (0 °C) emulsion of acrolein dimethyl acetal (**23.2**) (7.36 g, 72.06 mmol) in water (30.0 mL). The temperature of the mixture was kept below 14 °C, and cooling and stirring were continued for 25 min after the end of the addition. The cold bath was removed and $NaHCO_3$ (4.5 g, 42.4 mmol) and 1 M aqueous $Na_2S_2O_3$ (3.0 mL, 3.0 mmol) were added to the mixture, which was then saturated with NaCl and extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give the crude chlorohydrin, which was used directly in the next step.

Powdered NaOH (5.50 g, 137.5 mmol) was tipped into a stirred solution of the crude chlorohydrin in dry PhH (80.0 mL) (protection from moisture by $CaSO_4$ guard tube). The mixture was refluxed for 30 min, removed from the oil bath, stirred for 1 h, and filtered. Spinning band distillation of the filtrate gave **23.3** (6.171 g, 72%) as a pale yellow oil: FTIR ($CDCl_3$ cast) 2998, 1255 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.71-2.79 (m, 2 H), 3.04-3.10 (m, 1 H), 3.40 (s, 3 H), 3.42 (s, 3 H), 4.23 (d, J = 4.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 43.6 (t′), 51.2 (d′ or q′), 53.8 (d′ or q′), 54.5 (d′ or q′), 103.0 (d′); exact mass *m/z* calcd for $C_5H_9O_3$ (M - H) 117.0552, found 117.0553.

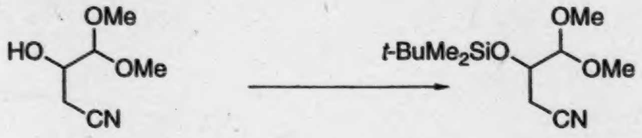
3-Hydroxy-4,4-dimethoxybutanenitrile (18).



23.3 **23.4**

A solution of KCN (414 mg, 6.36 mmol) in water (4 mL) was added to a stirred solution of epoxide **23.3** (501 mg, 4.24 mmol) in EtOH (10 mL).[30] Stirring was continued for 24 h, by which time all the starting material had reacted (TLC control, silica, 1:3 EtOAc-hexanes). The solvent was evaporated and the residue was filtered through a pad (5 x 4 cm) of silica gel, using CH_2Cl_2. Evaporation of the filtrate gave **23.4** (469 mg, 76%) as a thick, colorless oil: FTIR (neat film) 3700-3200, 2252 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.49-2.68 (m, 2 H), 3.10 (br s, 1 H), 3.45 (s, 3 H), 3.46 (s, 3 H), 3.83-3.86 (m, 1 H), 4.25 (d, *J* = 5.6, 1 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 20.8 (t′), 55.2 (q′), 56.1 (q′), 67.5 (d′), 105.4 (d′), 117.6 (s′); exact mass *m/z* calcd for $C_6H_{10}NO_3$ 144.0661 (M - H), found 144.0660.

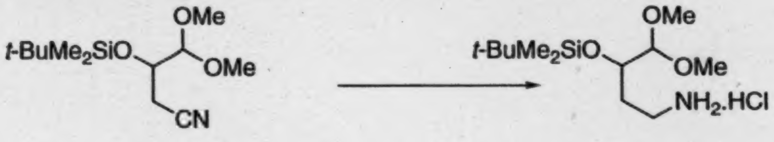
3-[[Dimethyl(1,1-dimethylethyl)silyl]oxy]-4,4-dimethoxybutanenitrile (23.5).



23.4 **23.5**

t-$BuMe_2SiCl$ (2.252 g, 14.9 mmol) and DMAP (36.5 mg, 0.30 mmol) were tipped into a stirred and cooled (0 °C) solution of nitrile **23.4** (1.083 g, 7.469 mmol) and Et_3N (1.35 mL, 10.4 mmol) in dry CH_2Cl_2 (15 mL). Stirring at 0 °C was continued for 15 min; the mixture was allowed to warm to room temperature, and was then refluxed for 12 h. Evaporation of the solvent and flash chromatography of the residue over silica gel (3.5 x 20 cm), using 1:3 EtOAc-hexanes, gave **23.5** [1.272 g, 65% (or 81%, after correction for recovered **23.4** (0.214 g)] as a colorless oil: FTIR ($CDCl_3$ cast) 2250 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.12 (s, 3 H), 0.15 (s, 3 H), 0.92 (s, 9 H), 2.50-2.63 (m, 2 H), 3.45 (s, 3 H), 3.48 (s, 3 H), 3.85-3.89 (m, 1 H), 4.19 (d, $J = 4.9$ Hz, 1 H); ^{13}C NMR ($CDCl_3$, 50.5 MHz) δ -5.0 (q′), -4.6 (q′), 18.0 (s′), 21.8 (t′), 25.6 (q′), 56.5 (q′), 56.6 (q′), 69.8 (d′), 106.7 (d′), 118.0 (s′); exact mass (HR electrospray) *m/z* calcd for $C_{12}H_{25}NNaO_3Si$ 282.1501, found 282.1496.

3-[[Dimethyl(1,1-dimethylethyl)silyl]oxy]-4,4-dimethoxybutanamine hydrochloride (23.6).



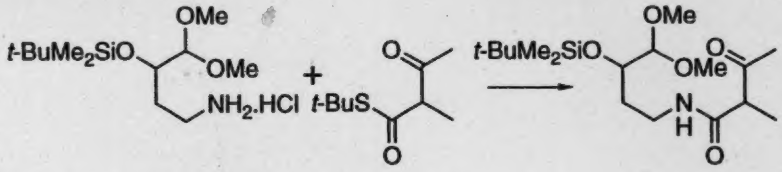
23.5 **23.6**

Adams catalyst (PtO_2) (82 mg) was suspended in dry EtOH (40 mL, distilled from Mg/I_2), and a solution of nitrile **23.5** (934 mg, 3.60 mmol) in dry EtOH (10 mL) was added to the suspension, followed by bench $CHCl_3$ (1.85 mL). The mixture was shaken under H_2 (50 psi, Parr bottle) at room temperature for 24 h. The catalyst was filtered off and the filtrate was evaporated. The residue was kept under oil pump vacuum for 24 h to give **23.6** (1.08 g, 100%). Recrystallization from CH_2Cl_2-petroleum ether (bp 60-70 °C) gave **23.6** as white flakes in quantitative yield. The material had: mp 118-122 °C; FTIR (USCOPE) 3460, 3300-2500, 2049 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.10 (s, 3 H), 0.11 (s, 3 H), 0.89 (s, 9 H), 1.72 (br s, 2 H), 1.93-2.14 (m, 2 H), 3.10-3.23 (m, 2 H), 3.44 (s, 3 H), 3.52 (s, 3 H), 3.85-3.89 (m, 1 H), 4.18 (d, J = 3.9 Hz, 1 H), 8.20 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 100.5 MHz) δ -4.7 (q'), 18.0 (s'), 25.8 (q'), 29.4 (t'), 36.0 (t'), 56.4 (q'), 57.4 (q'), 71.0 (d'), 107.6 (d'); exact mass (HR electrospray) *m/z* calcd for $C_{12}H_{30}NO_3Si$ 264.1995, found 264.1990.

The yield in this experiment varied between 60% and 75%.

In the above case, the recovered catalyst appeared to be very active, and burned on exposure to air.

***N*-[3-[[Dimethyl(1,1-dimethylethyl)silyl]oxy]-4,4-dimethoxybutyl]-2-methyl-3-oxobutanamide (25.3).**

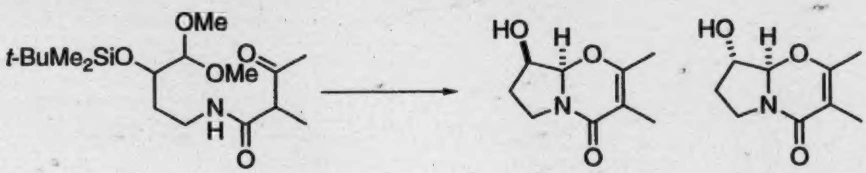


23.6 **25.2** **25.3**

Et_3N (0.26 mL, 1.88 mmol) was added to a stirred solution of amine hydrochloride **23.6** (282 mg, 0.94 mmol) and thioester **25.2**^{37} (177 mg, 0.94 mmol) in dry THF (2 mL). $AgOSO_2CF_3$ (488 mg, 1.88 mmol) was tipped into the mixture. After 40 min, the reaction was complete (TLC control, silica, 1:1 EtOAc-hexanes). The brown mixture was poured into a small volume of hexanes above a column of silica gel (2 x 15 cm), and the column was developed in the standard manner for flash chromatography, using 1:1 EtOAc-hexanes, to give **25.3** (251 mg, 79%) as a pale yellow oil: FTIR ($CDCl_3$ cast) 3500-3150, 1722, 1644 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.07 (s, 3 H), 0.08 (s, 3 H), 0.89 (s, 9 H), 1.36 (d, J = 7 Hz, 3 H), 1.68-1.83 (m, 2 H), 2.22 (s, 3 H), 3.28-3.38 (m, 3 H), 3.42 (d, J = 0.6 Hz, 3 H), 3.44 (d, J = 0.7 Hz, 3 H), 3.70-3.76 (m, 1 H), 4.15 (dd, J = 7.0, 0.7 Hz, 1 H), 6.45 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) (mixture of rotamers) δ -4.9 (q′),

-5.0 (q′), 14.2 (q′), 14.3 (q′), 18.1 (s′), 25.8 (q′), 28.35 (q′), 28.39 (q′), 31.5 (t′), 36.1 (t′), 55.25 (q′), 55.30 (q′), 56.0 (q′), 56.2 (q′), 71.68 (d′), 71.73 (d′), 107.6 (d′), 169.1 (s′), 169.2 (s′), 207.0 (s′); exact mass (HR electrospray) *m/z* calcd for $C_{17}H_{35}NNaO_5Si$ 384.2182, found 384.2187.

(8α,8aα)-6,7,8,8a-Tetrahydro-8-hydroxy-2,3-dimethyl-4*H*-pyrrolo[2,1-b][1,3]oxazine-4-one (27.1b) and (8α,8aβ)-6,7,8,8a-Tetrahydro-8-hydroxy-2,3-dimethyl-4*H*-pyrrolo[2,1-b][1,3]oxazine-4-one (27.1a).

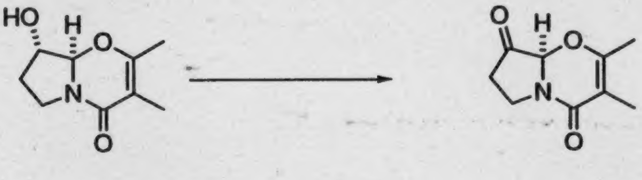


25.3 **27.1a** **27.1b**

Aqueous CF_3CO_2H (50%, 2.0 mL) was added to a stirred solution of β-keto amide **25.3** (187.5 mg, 0.518 mmol) in $CHCl_3$ (4 mL). Stirring was continued for 48 h and the solvent was then evaporated. The residue was kept under oil pump vacuum for 24 h, after which time a white solid was obtained. Flash chromatography over silica gel (1.2 x 20 cm), using 1:1 EtOAc-Et_2O and then 2:9:9 MeOH-EtOAc-Et_2O, gave the less polar isomer **27.1b** (38.4 mg, 37%) and the more polar isomer **27.1a** (36.3 mg, 35%) as white crystalline solids. Compound **27.1b** had: mp 104-106 °C; FTIR ($CDCl_3$ cast) 3600-3100, 1645, 1450 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (d, *J* = 0.5 Hz, 3 H), 1.87-1.98 (m, 1 H), 1.95 (d, *J* = 0.6 Hz, 3 H), 2.21-2.29 (m, 2 H), 3.59-3.71 (m, 2 H), 4.45-4.52 (m, 1 H), 5.07 (d, *J* = 3.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 10.2 (q′), 16.7 (q′), 30.0 (t′), 41.7 (t′), 75.3 (d′), 92.1 (d′), 106.7 (s′), 159.8 (s′), 163.1 (s′); exact mass *m/z* calcd for $C_9H_{13}NO_3$ 183.0895, found 183.0897. The structure was confirmed by X-ray analysis (see Appendix).

Compound **27.1a** had: mp 118-120 °C; FTIR ($CDCl_3$ cast) 3600-3100, 1651, 1458 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.80 (d, *J* = 0.8 Hz, 3 H), 1.94-2.14 [m including d at δ 1.98 (*J* = 0.9 Hz), 5 H in all], 2.44-2.48 (m, 1 H), 3.52-3.60 (m, 1 H), 3.68-3.78 (m, 1 H), 4.42-4.47 (m, 1 H), 5.24 (d, *J* = 3.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 10.2 (q′), 16.8 (q′), 29.3 (t′), 41.7 (t′), 70.6 (d′), 87.6 (d′), 106.9 (s′), 159.0 (s′), 162.9 (s′); exact mass *m/z* calcd for $C_9H_{13}NO_3$ 183.0895, found 183.0896.

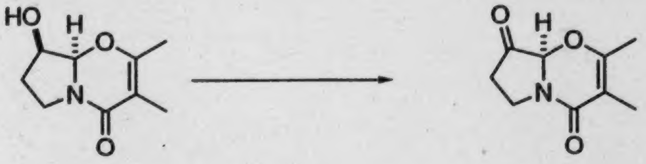
6,7-Dihydro-2,3-dimethyl-4*H*-pyrrolo[2,1-b][1,3]-oxazine-4,8(8a*H*)-dione (27.2) from less polar alcohol.



27.1b **27.2**

Dess-Martin periodinane (17.8 mg, 0.04 mmol) was added to a solution of the less polar isomer **27.1b** (5.9 mg, 0.032 mmol) in dry CH_2Cl_2 (1 mL). The mixture was stirred for 2 h, and then applied directly to a silica gel column (0.8 x 13 cm) made up with 99:1 EtOAc-hexanes. Flash chromatography, using the same solvent, gave **27.2** (2.5 mg, 42%) as a colorless oil: FTIR ($CDCl_3$ cast) 1772, 1659 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 1.79 (d, J = 0.8 Hz, 3 H), 2.00 (d, J = 0.9 Hz, 3 H), 2.60-2.78 (m, 2 H), 3.52-3.62 (m, 1 H), 4.00-4.10 (m, 1 H), 5.06 (s, 1 H); ^{13}C NMR (CD_2Cl_2, 100.6 MHz) δ 10.3 (q′), 16.9 (q′), 34.1 (t′), 38.3 (t′), 82.2 (d′), 107.5 (s′), 160.0 (s′), 163.4 (s′), 204.9 (s′); exact *m/z* calcd for $C_9H_{11}NO_3$ 181.0739, found 181.0738.

6,7-Dihydro-2,3-dimethyl-4*H*-pyrrolo[2,1-b][1,3]-oxazine-4,8(8a*H*)-dione (27.2) from more polar alcohol.

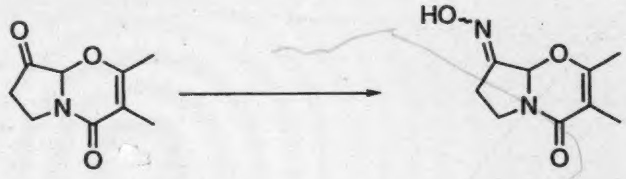


27.1a **27.2**

N-Methylmorpholine *N*-oxide (8.64 mg, 0.074 mmol), Pr_4NRuO_4 (0.9 mg, 0.0026 mmol) and crushed 4Å molecular sieves (25 mg) were added in succession to a stirred solution of the more polar isomer **27.1a** (9.0 mg, 0.049 mmol) in dry 1:1 CH_2Cl_2-MeCN (0.5 mL). Stirring was continued for 1.5 h, and the mixture was then loaded onto a silica gel column (0.8 x

14 cm) made up with 99:1 EtOAc-hexanes. Flash chromatography, using the same solvent, gave **27.2** [4.2 mg, 46% or 84% after correction for recovered starting material (4.0 mg)] as a colorless oil, spectroscopically identical to the compound obtained from the other isomer.

6,7-Dihydro-2,3-dimethyl-4*H*-pyrrolo[2,1-b][1,3]-oxazine-4,8(8a*H*)-dione 8-oxime (19.2).

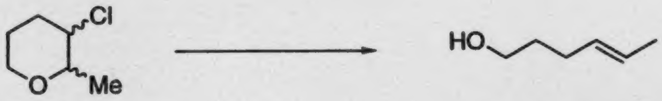


27.2 **19.2**

A solution of $NH_2OH.HCl$ (8.8 mg, 0.127 mmol) and AcONa (17.6 mg, 0.129 mmol) in water (0.2 mL) was added to a stirred solution of ketone **27.2** (4.4 mg, 0.024 mmol) in EtOH (0.2 mL). Stirring was continued for 3.5 h, and the solvent was then evaporated. Flash chromatography of the residue over silica gel (0.8 x 20 cm), using 99:1 EtOAc-hexanes, gave the less polar *E* oxime **15a** (3.5 mg, 74%) and the more polar *Z* oxime **15b** (0.3 mg, 6.3%) as white solids. Compound **15a** had: mp 176-178 °C and then solidifies, but does not melt again; FTIR (CH_2Cl_2 cast) 3600-2950, 1643 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 1.79 (d, J = 0.8 Hz, 3 H), 1.98 (d, J = 0.9 Hz, 3 H), 2.74-2.98 (m, 2 H), 3.41-3.50 (m, 1 H), 3.94-4.04 (m, 1 H), 5.59 (d, J = 1.5 Hz, 1 H), 8.40 (s, 1 H); ^{13}C NMR (CD_2Cl_2, 100.6 MHz) δ 10.3 (q′), 16.9 (q′), 23.9 (t′), 41.9 (t′), 84.5 (d′), 107.4 (s′), 158.3 (s′), 160.7 (s′), 163.3 (s′); exact mass *m/z* calcd for $C_9H_{12}N_2O_3$ 196.0848, found 196.0843.

Compound **15b** had: FTIR (CD_2Cl_2 cast) 3500-3000, 1640 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 1.78 (d, *J* = 0.9 Hz, 3 H), 1.98 (d, *J* = 0.8 Hz, 3 H), 2.62-2.71 (m, 1 H), 2.78-2.90 (m, 1 H), 3.26-3.34 (m, 1 H), 4.00-4.08 (m, 1 H), 5.87 (d, *J* = 1.4 Hz, 1 H), 7.56 (s, 1 H); ^{13}C NMR (CD_2Cl_2, 100.6 MHz) δ 10.4, 17.0, 27.4, 41.7, 80.1, 107.2, 157.5, 160.5, 162.8; exact mass *m/z* calcd for $C_9H_{12}N_2O_3$ 196.0848, found 196.0849. The mp was not measured.

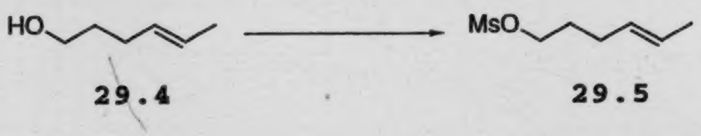
(*E*)-4-Hexenol (29.4).[41]



29.2 **29.4**

Freshly cut Na (2.43 g, 105 mmol) was powdered by heating in xylenes (dried over 4Å molecular sieves, 75 mL) at 120 °C with stirring. The resulting suspension was cooled, and the Na powder was washed with dry Et_2O under N_2 and then covered with dry Et_2O (20 mL), the washings being removed each time by suction through a cannula. A few drops of a solution of 3-chloro-2-methyl-tetrahydropyran[41b] (**Hazard warning**) (6.00 g, 42.3 mmol) in dry Et_2O (20 mL) was added to the Na with vigorous magnetic stirring. After a few min, a vigorous reaction occurred and a purple mixture was formed. The remaining pyran solution was added at such a rate as to maintain gentle reflux. The blue mixture was left for 28 h at room temperature, and the excess of Na was carefully destroyed (N_2 atmosphere) with wet Et_2O, followed by water. The ether layer was separated and the aqueous layer was extracted with Et_2O. The combined organic extracts were washed successively with 5% aqueous HCl (50 mL) and brine, and then dried ($MgSO_4$). The solvent was removed by distillation at 1 atm, and the oily residue was distilled to yield **29.4** (3.99 g, 94%) as a 95:5 mixture of *E* and *Z* isomers, bp 85-90 °C (water pump vacuum). Spinning band distillation at 158-159 °C (1 atm) gave pure (GC-MS) (*E*)-hex-4-enol as a colorless liquid in 20% yield: FTIR (neat film) 3600-3100 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.56-1.66 [m containing d at δ 1.63 (J = 6.2 Hz), 5 H in all], 1.90 (s, 1 H), 2.01-2.09 (m, 2 H), 3.61 (t, J = 6.5 Hz, 2 H), 5.37-5.50 (m, 2 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 17.8 (q′), 28.8 (t′), 32.4 (t′), 62.4 (t′), 125.4 (d′), 130.6 (d′); exact mass *m/z* calcd for $C_6H_{12}O$ 100.0888, found 100.0887.

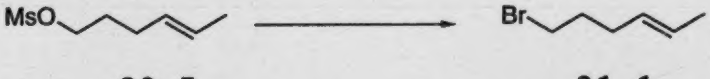
Methanesulfonic acid (*E*)-4-hexenyl ester (29.5).[44]



$MeSO_2Cl$ (1.81 mL, 23.3 mmol) was added dropwise to a stirred and cooled (0 °C) solution of (*E*)-4-hexenol **(29.4)**

(1.915 g, 19.2 mmol) and Et_3N (3.33 mL, 23.2 mmol) in dry THF (400.0 mL). Stirring at 0 °C was continued for 40 min, the cold bath was removed, and stirring was continued for 30 min. The mixture was quenched with water (25 mL) and diluted with CH_2Cl_2 (100 mL). The organic phase was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$), and evaporated to give crude **29.5** (3.409 g, 100%) as a pale yellow oil, suitable for the next step. Pure mesylate, obtained by flash chromatography over silica gel (3.5 x 16 cm), using 1:4 EtOAc-hexanes had: FTIR ($CDCl_3$ cast) 1352, 1173 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.62-1.64 (m, 3 H), 1.74-1.82 (m, 2 H), 2.05-2.11 (m, 2 H), 2.98 (s, 3 H), 4.19 (dt, J = 6.5, 0.94 Hz, 2 H), 5.31-5.41 (m, 1 H), 5.42-5.52 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 17.8 (q′), 28.2 (t′), 28.8 (t′), 37.3 (q′), 69.5 (t′), 126.6 (d′), 129.1 (d′); exact mass m/z calcd for $C_7H_{14}O_3S$ 178.0664, found 178.0663.

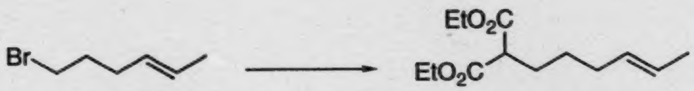
(*E*)-6-Bromo-2-hexene (31.1).[44]



29.5 **31.1**

Anhydrous LiBr (dried overnight at 110 °C under oil pump vacuum, 2.94 g, 33.8 mmol) was tipped into a stirred solution of crude mesylate **29.5** (2.008 g, 11.28 mmol) in dry THF (40.0 mL). The resulting solution was refluxed for 3.5 h, by which time all starting material had been consumed (TLC control, silica gel, 1:4 EtOAc-hexanes). The mixture was cooled and added to pentane (200 mL), washed with water, dried ($MgSO_4$) and evaporated. The residue was distilled to give **31.1** (1.605 g), as a colorless, acrid liquid: bp 165 °C (760 mm Hg); FTIR ($CDCl_3$ cast) 1779, 1740 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.63-167 (m, 3 H), 1.86-1.94 (m, 2 H), 2.10-2.70 (m, 2 H), 3.40 (t, J = 6.8 Hz, 2 H), 5.32-5.42 (m, 1 H), 5.45-5.55 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 17.9 (q'), 30.9 (t'), 32.5 (t'), 33.3 (t'), 126.4 (d'), 129.2 (d'); exact mass m/z calcd for $C_6H_{11}{}^{79}Br$ 162.00446, found 162.0044.

Diethyl (*E*)-2-(4-Hexenyl)propanedioate (31.2).[45,46]

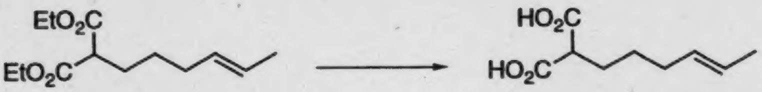


31.1 **31.2**

(*E*)-6-Bromohex-2-ene (**31.1**) (1.515 g, 9.30 mmol) in dry EtOH (5.0 mL) was added dropwise to a stirred solution of $NaCH(CO_2Et)_2$ [prepared from $CH_2(CO_2Et)_2$ (1.53 mL, 11.2 mmol) and Na (257 mg, 11.6 mmol) in dry EtOH (6.0 mL) at 50 °C]. The resulting mixture was refluxed under Ar for 3 h, and then cooled. Most of the solvent was evaporated, and the residue was taken up in pentane (100 mL), washed with water, dried ($MgSO_4$), evaporated, and fractionally distilled to give **31.2** (1.636 g, 72%) as a colorless oil: bp 172 °C (water-pump

vacuum); FTIR ($CDCl_3$ cast) 1734 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.24 (t, J = 7.2 Hz, 6 H), 1.31-1.40 (m, 2 H), 1.60-1.62 (m, 3 H), 1.87 (q, J = 7.9 Hz, 2 H), 1.94-2.02 (m, 2 H), 3.29 (t, J = 7.6 Hz, 1 H), 4.17 (q, J = 7.1 Hz, 4 H), 5.30-5.47 (m, 2 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 14.1 (q′), 17.9 (q′), 27.2 (t′), 28.2 (t′), 32.1 (t′), 51.9 (d′), 61.2 (t′), 125.5 (d′), 130.5 (d′), 169.5 (s′); exact mass m/z calcd for $C_{13}H_{22}O_4$ 242.1518, found 242.1516.

(*E*)-2-(4-Hexenyl)propanedioic acid (31.3).[46]

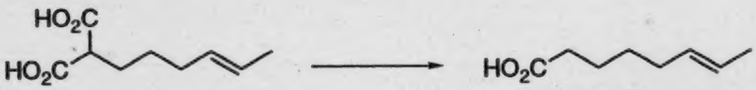


31.2 **31.3**

Diethyl (*E*)-hex-4-enylmalonate (**31.2**) (1.64 g, 6.76 mmol) was added to a stirred and cooled (0 °C) solution of KOH (1.51 g, 27.3 mmol) in water (26 mL), followed by sufficient EtOH (12 mL) to produce homogeneity. After 24 h, the solution was washed with CH_2Cl_2 and the aqueous layer was cooled (0 °C) and acidified to pH ca 2 (universal indicator) with concentrated HCl. The precipitated diacid **31.3** was extracted with Et_2O, and the combined extracts were dried ($MgSO_4$) and evaporated to give crude **31.3** (1.248 g, 99%), which was recrystallized from PhH to give pure **31.3** (739 mg, 59% recovery) as a white solid: mp 110-112 °C (lit.[46] 115-116 °C); FTIR (USCOPE) 3400-2400 cm^{-1}, 1709 cm^{-1}; ^1H NMR (DMSO-d_6, 360 MHz) δ 1.40-1.51 (m, 2 H), 1.65 (dd, J = 5.5, 0.6 Hz, 3

H), 1.91-1.97 (m, 2 H), 2.00-2.06 (m, 2 H), 3.44 (t, J = 7.4 Hz, 1 H), 5.33-5.51 (m, 2 H), 10.3-11.0 (br s, 2 H); ^{13}C NMR (DMSO-d_6, 50.3 MHz) δ 17.7 (q′), 26.7 (t′), 27.9 (t′), 31.7 (t′), 51.4 (d′), 124.8 (d′), 130.8 (d′), 170.9 (s′); exact mass m/z calcd for $C_9H_{14}O_4$ 186.0892, found 186.0888.

(*E*)-6-Octenoic acid (31.4).[46]

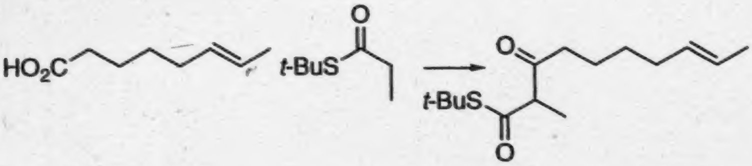


31.3 **31.4**

A round-bottomed 50-mL flask containing (*E*)-hex-4-enylmalonic acid (**31.3**) (943 mg, 5.07 mmol) was lowered into a preheated (155-160 °C) oil bath. After 5.5 h, the flask was cooled and its contents were dissolved in saturated aqueous $NaHCO_3$ (15 mL). The resulting solution was washed with Et_2O and the aqueous layer was acidified with concentrated HCl. The precipitated acid was extracted with Et_2O, and the combined extracts were dried ($MgSO_4$) and evaporated to give the crude acid. Flash chromatography over silica gel (2 x 20 cm), using 1:1 Et_2O-hexanes, gave **31.4** (676 mg, 93%) as a colorless oil: FTIR ($CDCl_3$ cast) 3600-2300, 1706 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35-1.48 (m, 2 H), 1.58-1.68 (m, 5 H), 1.96-2.04 (m, 2 H), 2.35 (t, J = 7.5 Hz, 2 H), 5.33-5.50 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 17.9 (q′), 24.1 (t′), 28.9 (t′), 32.1 (t′), 33.8 (t′), 125.3 (d′), 130.8 (d′), 179.5 (s′); exact mass m/z calcd for $C_8H_{14}O_2$

142.0994, found 142.0991.

S-(1,1-Dimethylethyl) (E)-2-Methyl-3-oxo-8-decenethioate (28.1).



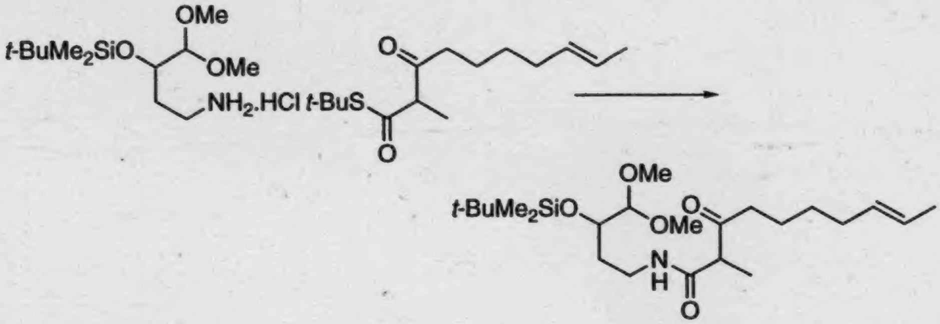
31.4 **30.1** **28.1**

1,1′-Carbonyldiimidazole (293 mg, 1.81 mmol) was tipped into a stirred and cooled (0 °C) solution of (E)-6-octenoic acid (**31.4**) (233 mg, 1.64 mmol) in dry THF (0.8 mL). Brisk evolution of gas occurred. When the reaction had subsided, the cold bath was removed and stirring was continued for 30 min.

In the meantime the lithium enolate of thioester **30.1**[49] was prepared by slow addition of LDA [made by dropwise addition of BuLi (2.5 M in hexanes, 1.97 mL, 4.92 mmol) to i-Pr_2NH (0.69 mL, 4.92 mmol) in dry THF (1.0 mL) at -78 °C, followed by warming to 0 °C (transfer to an ice bath) for 5 min, and recooling to -78 °C] to a stirred and cooled (-78 °C) solution of **30.1** (719 mg, 4.92 mmol) in THF (1 mL). After 15 min a yellow solution was obtained.

The imidazolide solution made in the first part of this experiment was cooled to -78 °C and transferred by cannula over 10 min into the stirred and cooled (-78 °C) enolate solution. Stirring at -78 °C was continued for 30 min, the cold bath was removed, and stirring was continued for a further 5 min. The reaction was quenched with saturated aqueous NH_4Cl (2 mL) and the mixture was diluted with Et_2O (30 mL). The aqueous layer was extracted with Et_2O, and the combined organic extracts were dried ($MgSO_4$) and evaporated to give a yellow oil. Flash chromatography over silica gel (2 x 24 cm), using with 1:24 Et_2O-petroleum ether (bp 60-70 °C) gave **28.1** (287.2 mg, 64%) as a colorless oil: FTIR ($CDCl_3$ cast) 1725, 1673 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.28-1.37 [m, containing d at δ 1.31 (J = 7.0 Hz), 5 H in all), 1.47 (s, 9 H), 1.51-1.63 (m, 2 H), 1.61-1.63 (m, 3 H), 1.92-2.02 (m, 2 H), 2.41-2.62 (m, 2 H), 3.64 (q, J = 7.0 Hz, 1 H), 5.32-5.48 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 13.5 (q′), 17.9 (q′), 23.1 (t′), 28.9 (t′), 29.6 (q′), 32.2 (t′), 41.1 (t′), 48.8 (s′), 62.1 (d′), 125.1 (d′), 130.9 (d′), 197.1 (s′), 204.9 (s′); exact mass *m/z* calcd for $C_{15}H_{26}O_2S$ 270.1653, found 270.1645.

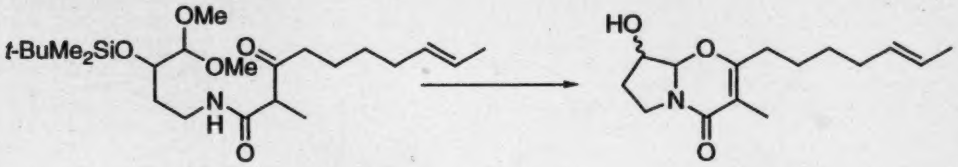
(*E*)-*N*-[3-[[Dimethyl(1,1-dimethylethyl)silyl]oxy]-4,4-dimethoxybutyl]-2-methyl-3-oxo-8-decenamide (32.1).



23.6 **28.1** **32.1**

$AgOSO_2CF_3$ (224 mg, 0.864 mmol) was tipped into a stirred mixture of amine hydrochloride **23.6** (129 mg, 0.432 mmol), dry Et_3N (0.12 mL, 0.864 mmol) and β-keto thioester **28.1** (116.6 mg, 0.432 mmol) in dry THF (5 mL). The reaction was over in ca 20 min (TLC control, silica, 1:1 EtOAc-hexanes). The mixture was loaded onto a dry silica gel column (2 x 18 cm) and flash chromatography, using 1:1 EtOAc-hexanes, gave **32.1** (147.4 mg, 90%) as a thick, pale yellow oil: FTIR ($CDCl_3$ cast) 3450-3150, 1720, 1643 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.08 (s, 3 H), 0.09 (s, 3 H), 0.90 (s, 9 H), 1.24-1.33 (m, 2 H), 1.35 (dd, *J* = 7.1, 0.6 Hz, 3 H), 1.52-1.61 (m, 2 H), 1.63 (d, *J* = 4.7 Hz, 3 H), 1.67-1.83 (m, 2 H), 1.94-1.99 (m, 2 H), 2.47-2.61 (m, 2 H), 3.31-3.40 (m, 3 H), 3.43 (s, 3 H), 3.44 (d, *J* = 1.3 Hz, 3 H), 3.72 (ddd, *J* = 6.3, 4.9, 1.4 Hz, 1 H), 4.12 (d, *J* = 4.8 Hz, 1 H), 5.34-5.46 (m, 2 H), 6.47 (d, *J* = 5.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) (mixture of rotamers) δ -4.8 (q'), -4.5 (q'), 14.7 (q'), 14.8 (q'), 17.9 (q'), 18.2 (s'), 22.9 (t'), 25.9 (q'), 28.9 (t'), 31.7 (t'), 32.3 (t'), 36.1 (t'), 41.26 (t'), 41.29 (t'), 54.58 (q'), 54.64 (q'), 55.94 (d'), 56.2 (q'), 71.66 (d'), 71.71 (d'), 107.6 (d'), 125.2 (d'), 130.9 (d'), 126.29 (s'), 169.35 (s'), 209.4 (s'); exact mass *m/z* calcd for $C_{23}H_{45}NO_5Si$ 443.3067, found 443.3060.

(*E*)-2-(5-Heptenyl)-6,7,8,8a-tetrahydro-8-hydroxy-3-methyl-4*H*-pyrrolo[2,1-b][1,3]oxazine-4-one (5.3a,b).

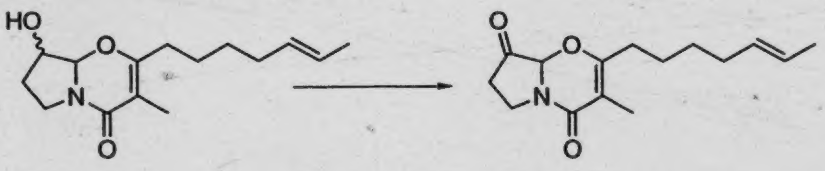


32.1 **5.3a,b**

Aqueous CF_3CO_2H (50%, 1.0 mL) was added to a stirred solution of β-keto amide **32.1** (224 mg, 0.50 mmol) in $CHCl_3$ (2 mL). Stirring was continued for 36 h, by which time the starting material had been consumed (TLC control, silica, 1:1 EtOAc-hexanes). The solvent was evaporated and the residue was left under oil pump vacuum for 24 h, to obtain a mixture of diastereoisomers **5.3** (133.5 mg, ca 100%) as a white solid. Flash chromatography over silica gel (2 x 20 cm), using 1:24 MeOH-Et_2O, gave the less polar diastereoisomer **5.3a** (61.4 mg, 45%) and the more polar diastereoisomer **5.3b** (56.4 mg, 42%) as colorless liquids. Compound **5.3a** had: FTIR ($CDCl_3$ cast) 3600-3100, 1643 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.30-1.43 (m, 2 H), 1.48-1.60 (m, 2 H), 1.61-1.66 (m, 3 H), 1.78 (s, 3 H), 1.87-2.01 (m, 3 H), 2.15-2.33 (m, 3 H), 2.97 (br s, 1 H), 3.56-3.71 (m, 2 H), 4.42-4.46 (m, 1 H), 5.03 (d, *J* = 3.7 Hz, 1 H), 5.35-5.47 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 10.0 (q′), 17.9 (q′), 26.3 (t′), 29.1 (t′), 30.1 (t′), 30.5 (t′), 32.2 (t′), 41.8 (t′), 75.2 (d′), 92.3 (d′), 106.4 (s′), 125.2 (d′), 130.9 (d′), 163.4 (s′), 163.5 (s′); exact mass *m/z* calcd for $C_{15}H_{23}NO_3$ 265.1678, found 265.1675.

Compound **5.3b** had: FTIR ($CDCl_3$ cast) 3600-3050, 1737, 1645 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.34-1.44 (m, 2 H), 1.50-1.61 (m, 2 H), 1.65 (dd, *J* = 4.9, 1.2 Hz, 3 H), 1.79 (s, 3 H), 1.92-2.13 (m, 4 H), 2.19-2.26 (m, 1 H), 2.30-2.38 (m, 1 H), 2.50 (br s, 1 H), 3.55-3.64 (m, 1 H), 3.66-3.78 (m, 1 H), 4.30-4.90 (m, 1 H), 5.20 (d, *J* = 3.5 Hz, 1 H), 5.33-5.49 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 10.0 (q′), 17.9 (q′), 26.3 (t′), 29.1 (t′), 29.3 (t′), 30.5 (t′), 32.2 (t′), 41.7 (t′), 70.6 (d′), 87.7 (d′), 106.8 (s′), 125.3 (d′), 130.8 (d′), 162.5 (s′), 163.1 (s′); exact mass *m/z* calcd for $C_{15}H_{23}NO_3$ 265.1678, found 265.1670.

(*E*)-2-(5-Heptenyl)-6,7-dihydro-3-methyl-4*H*-pyrrolo[2,1-b][1,3]oxazine-4,8(8a*H*)-dione (5.4) from less polar alcohol.

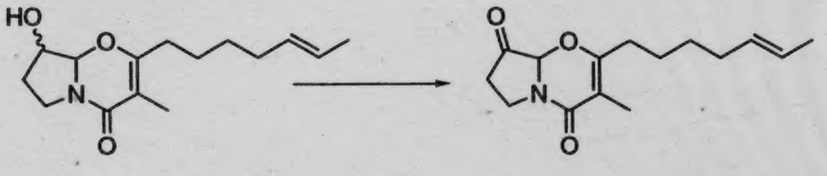


5.3a **5.4**

Dess-Martin periodinane (68.8 mg, 0.16 mmol) in dry CH_2Cl_2 (1 mL) was added to a stirred solution of the less polar alcohol **5.3a** (28.8 mg, 0.11 mmol) in dry CH_2Cl_2 (0.5 mL). Stirring was continued for 2 h (TLC control, silica, 3:1 EtOAc-hexanes), and the mixture was diluted with EtOAc (4 mL) and stirred for 5 min with saturated aqueous $NaHCO_3$ (2 mL) containing $Na_2S_2O_3$ (250 mg). More EtOAc (8 mL) was added, followed by water (4 mL). The aqueous layer was extracted with EtOAc, and the combined organic extracts were evaporated. Flash chromatography of the oily residue over silica gel (1 x 20 cm), using 3:1 EtOAc-hexanes, gave **5.4** (15.6 mg, 54%, or 99% after correction for recovered starting material (13.0 mg, 45%)) as a colorless liquid: FTIR ($CDCl_3$ cast) 1774, 1664 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.32-1.42 (m, 2 H), 1.51-1.61 (m, 2 H), 1.64 (dd, *J* = 3.8, 0.9 Hz, 3 H), 1.84 (s, 3 H), 1.94-2.06 (m, 2 H), 2.21-2.42 (m, 2 H), 2.62-2.83 (m, 2 H), 3.57-3.66 (m, 1 H), 4.08-4.17 (m, 1 H), 5.03 (s, 1 H), 5.32-5.48 (m, 2 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 10.0

(q′), 17.9 (q′), 26.2 (t′), 29.1 (t′), 30.5 (t′), 32.2 (t′), 33.7 (t′), 38.0 (t′), 81.7 (d′), 107.1 (s′), 125.3 (d′), 130.8 (d′), 163.3 (s′), 163.5 (s′), 204.4 (s′); exact mass *m/z* calcd for $C_{15}H_{21}NO_3$ 263.1521, found 263.1514.

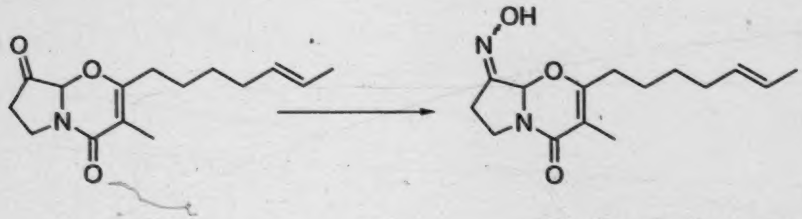
(*E*)-2-(5-Heptenyl)-6,7-dihydro-3-methyl-4*H*-pyrrolo[2,1-b][1,3]oxazine-4,8(8a*H*)-dione (5.4) from more polar alcohol.



5.3b **5.4**

N-Methylmorpholine *N*-oxide (12.3 mg, 0.105 mmol), Pr_4NRuO_4 (1.23 mg, 0.00350 mmol) and crushed 4Å molecular sieves (35.0 mg) were added in succession to a stirred solution of the more polar alcohol **5.3b** (18.6 mg, 0.07 mmol) in dry CH_2Cl_2 (1 mL). The mixture was stirred for 2 h and then loaded onto a silica gel column (1 x 15 cm) made up with 3:1 EtOAc-hexanes. Flash chromatography, using 3:1 EtOAc-hexanes, gave **5.4** [3.5 mg, 18% (23% after correction for recovered starting material (3.7 mg)] as a colorless liquid, spectroscopically identical to material obtained from the other isomer.

(*E*,*E*)- and (*E*,*Z*)-2-(5-Heptenyl)-6,7-dihydro-3-methyl-4*H*-pyrrolo[2,1-b][1,3]oxazine-4,8(8a*H*)-dione 8-oxime (1) and (36).



5.4 **32.2, 2.1**

A solution of $H_2NOH.HCl$ (29.9 mg, 0.43 mmol) and AcONa (60.8 mg, 0.44 mmol) in water (0.25 mL) was added to ketone **5.4** (22.6 mg, 0.09 mmol). EtOH (0.25 mL) was added to the mixture until turbidity disappeared, and the mixture was stirred for 3 h, by which time the starting material had been consumed (TLC control, silica, 3:1 EtOAc-hexanes). The solvent was evaporated and the residue was washed through a silica pad (1 x 4 cm), using EtOAc (15 mL). Evaporation of the filtrate gave a mixture of oxime isomers (23.8 mg, 99%) as a pale yellow solid. Flash chromatography over silica gel (1 x 20 cm), using 3:1 EtOAc-hexanes, gave the less polar *E*-isomer **2.1** (17.9 mg, 74%) and the *Z* isomer **32.2** (4.9 mg, 20%) as white solids. The ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) spectra of **2.1** were the same as those reported; the compound had: mp 146-149 °C.

Compound **32.2** (*Z* isomer) had: mp 138.5-140 °C; FTIR (CH_2Cl_2 cast) 3600-3050, 1641 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 1.35-1.44 (m, 2 H), 1.55-1.60 (m, 2 H), 1.63 (d, J = 3.6 Hz, 3 H), 1.78 (s, 3 H), 1.96-2.02 (m, 2 H), 2.17-2.25 (m, 1 H), 2.32-2.39 (m, 1 H), 2.63-2.70 (m Hz, 1 H), 2.79-2.90 (m, 2 H), 3.26-3.36 (m, 1 H), 3.98-4.07 (m, 1 H), 5.38-5.45 (m, 2 H), 5.81 (d, *J* = 1.3 Hz, 1 H), 7.66 (s, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 10.1 (q'), 17.9 (q'), 26.3 (t'), 27.1 (t'), 29.0 (t'), 30.5 (t'), 32.2 (t'), 41.4 (t'), 79.8 (d'), 106.9 (s'), 125.2 (d'), 130.9 (d'), 156.9 (s'), 163.2 (s'), 164.0 (s') (the spectrum showed a trace of the *E* isomer **2.1**); exact mass *m/z* calcd for $C_{15}H_{22}N_2O_3$ 278.1631, found 278.1624.

A sample (4.9 mg) of the *Z* oxime **32.2** was stored for 51 h in $CDCl_3$. Evaporation of the solvent and flash chromatography of the residue over silica gel, using 3:1 EtOAc-hexanes, gave **2.1** (2.2 mg, 44%) and **32.2** (2.1 mg, 42%).

The following Tables show the NMR data for the natural and synthetic brevioxime.

Table 1 (^{1}H NMR spectrum of brevioxime)

Natural	**Natural**	**Synthetic**	**Synthetic**
$CDCl_3$, 300 MHz		$CDCl_3$, 400 MHz	
1.35	m, 2 H	1.35-1.43	m, 2 H
1.55	m, 2 H	1.50-1.62	m, 2 H
1.63	d, J = 2.4 Hz, 3 H	1.63	d, J = 2.3 Hz, 3 H
1.83	s, 3 H	1.83	s, 3 H
1.98	m, 2 H	1.94-2.04	m, 2 H
2.28	m, 2 H	2.20-2.40	m, 2 H
2.88	m, 2 H	2.78-2.99	m, 2 H
3.47	m, 1 H	3.44-3.53	m, 1 H
4.05	m, 1 H	4.02-4.13	m, 1 H
5.38	m, 2 H	5.33-5.49	m, 2 H
5.50	s, 1 H	5.56	s, 1 H
8.02	s, 1 H	7.95	s, 1 H

Table 2 (^{13}C NMR spectrum of brevioxime)

Natural	**Synthetic**
$CDCl_3$, 75 MHz	$CDCl_3$, 100 MHz
10.0	10.1
17.8	18.0
23.6	23.8
26.2	26.4
29.1	29.3
30.5	30.7
32.1	32.3
41.5	41.7
84.0	84.2
106.9	107.1
125.1	125.3
130.8	131.0
158.0	158.4
163.2	163.3
163.6	163.8

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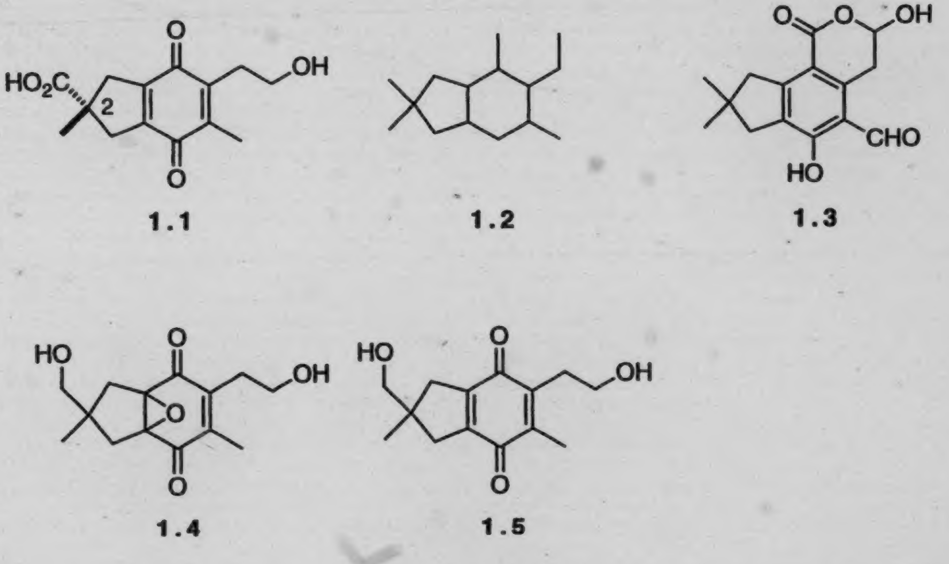
CHAPTER 2

SYNTHESIS OF (±)-PURAQUINONIC ACID

Introduction

Puraquinonic acid (**1.1**) is a fungal metabolite produced by cultures of *Mycena pura*.[1] The compound, which is optically active, with $[\alpha]_D^{22}$ +1, induces differentiation of HL-60 cells (human promyelocytic leukemia). This is an important property since there is evidence[2] that induction of cell differentiation leads to suppression of cell proliferation. Puraquinonic acid, may therefore serve as a lead compound in the design of drugs to treat leukemia.

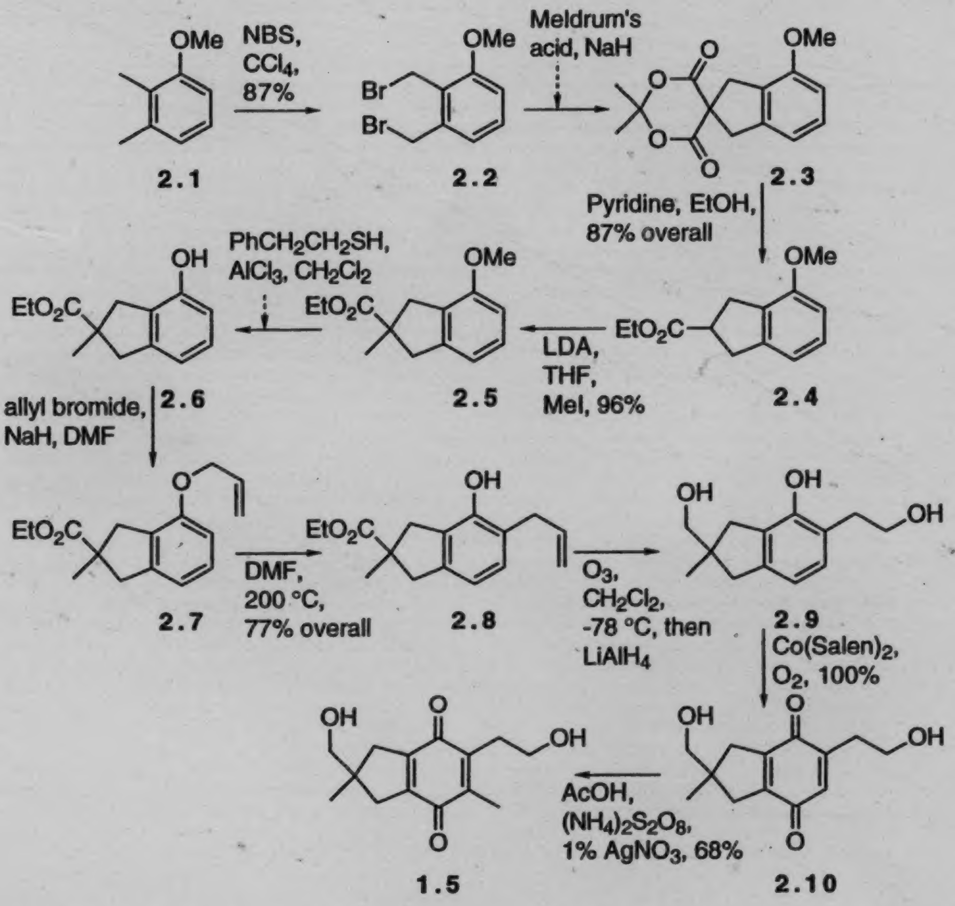


Scheme 1

Puraquinonic acid is a norilludalane sesquiterpene, illudalanes being natural products with the skeleton **1.2**, such as illudalic acid (**1.3**).[3] Two compounds, **1.4** (2,9-epoxydeliquinone) and **1.5** (deliquinone), closely related to puraquinonic acid have also been isolated,[4] but their biological properties do not appear to have been examined.

Synthesis of (±)-deliquinone

Prior to our own publications no synthetic work on puraquinonic acid or its relatives (**1.4** and **1.5**) had been reported, but recently, Kraus and Choudhury described[5] a short synthesis of (±)-deliquinone. 2,3-Dimethylanisole


OMe
NBS, CCl4, 87%
Br
Br
Meldrum's acid, NaH
O
2.1
2.2
2.3
Pyridine, EtOH, 87% overall
PhCH2CH2SH, AlCl3, CH2Cl2
OH
EtO2C
LDA, THF, MeI, 96%
2.4
2.5
2.6
allyl bromide, NaH, DMF
2.7
DMF, 200 °C, 77% overall
2.8
O3, CH2Cl2, -78 °C, then LiAlH4
2.9
Co(Salen)2, O2, 100%
2.10
AcOH, (NH4)2S2O8, 1% AgNO3, 68%
1.5

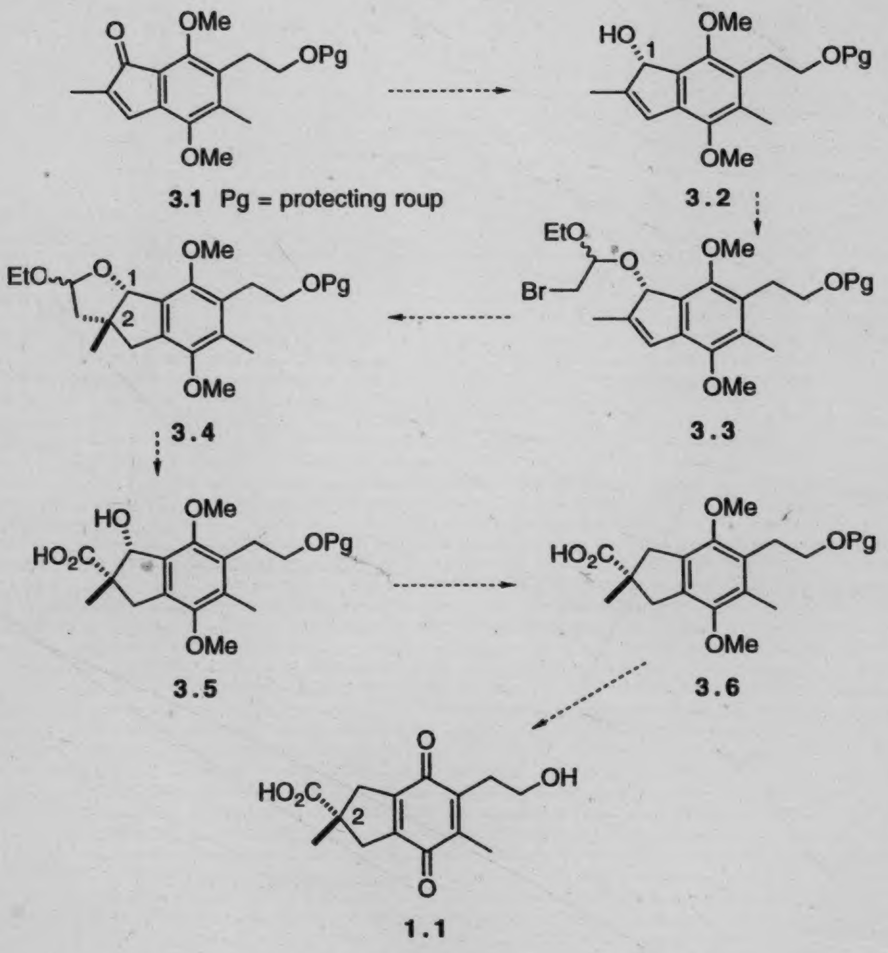
Scheme 2

(**2.1**) was subjected to double benzylic bromination, and then reaction with the sodium salt of Meldrum's acid afforded the spiro compound **2.3**. Next, treatment with EtOH and pyridine gave the ethyl ester **2.4**, which was then methylated. Removal of the *O*-methyl group and *O*-allylation now set the stage for a Claisen rearrangement, which afforded phenol **2.8**. Ozonolytic double bond cleavage and hydride reduction of the ozonide produced the phenolic alcohol **2.9**. This was oxidized to the corresponding quinone (**2.10**) and, finally, addition of a methyl radical under oxidative conditions introduced the last carbon required, giving (±)-deliquinone **1.5**.

Previous exploratory studies on the synthesis of (±)-puraquinonic acid done in this laboratory

Extensive exploratory work had been done in this laboratory by M. Sannigrahi[6] on the synthesis of puraquinonic acid (**1.1**), and, eventually, a promising route emerged.

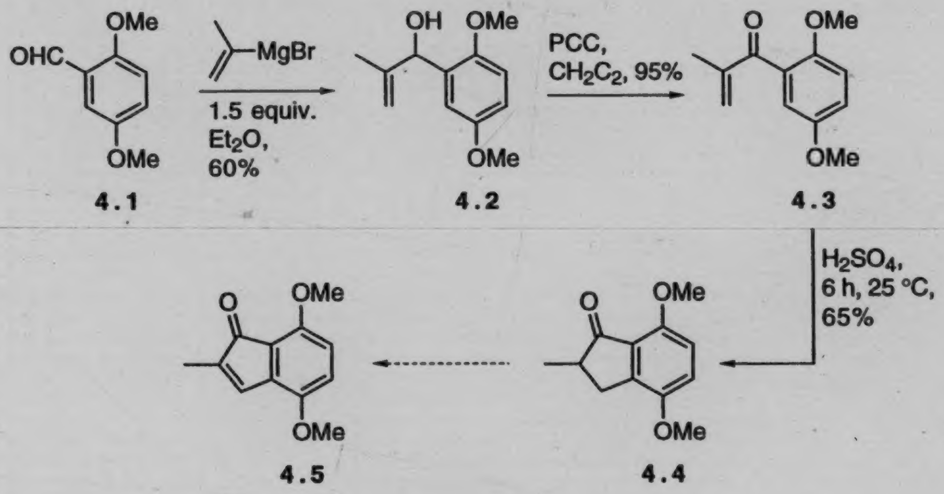
The approach was based on the radical cyclization of a Stork bromoacetal (Scheme 3, **3.3** → **3.4**). According to the rules for ring closure[7] a *cis*-fused product must form, so that the stereochemistry at C(1) in **3.2** would control the stereochemistry at C(2) in **3.4**. It was planned to degrade the heterocyclic ring in **3.4** (**3.4** → **3.5**) and then to remove the remaining hydroxyl by Barton deoxygenation (**3.5** → **3.6**). Removal of the remaining protecting group and oxidation of the benzene ring would then afford puraquinonic acid (**1.1**). The starting alcohol (**3.2**) was expected to be available from


3.1 Pg = protecting roup
3.2
3.3
3.4
3.5
3.6
1.1
OMe
OPg
HO
1
EtO
Br
2
HO$_2$C
OH
O

Scheme 3

the corresponding ketone (**3.1**) by use of one of the many methods for asymmetric ketone reduction.[8]

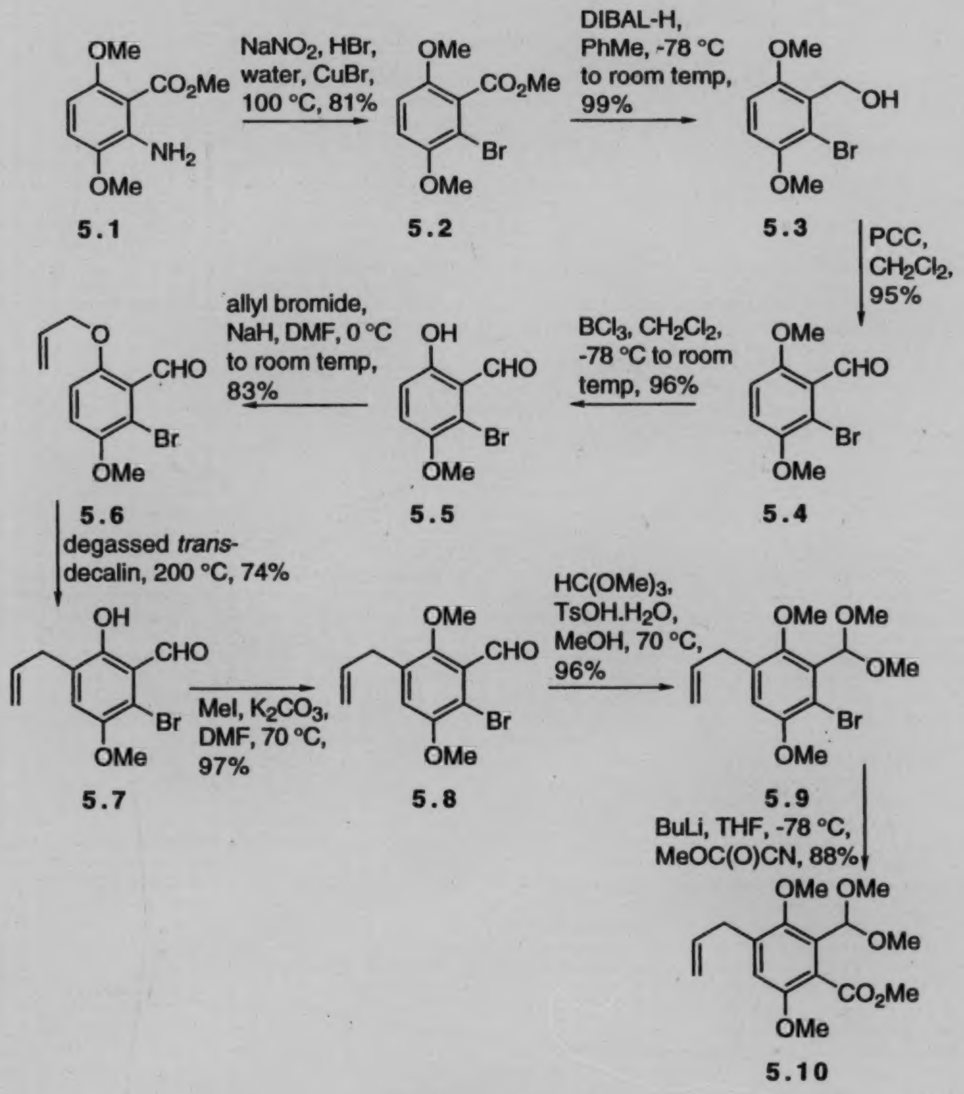
In order to implement the plan summarized in Scheme 3, the first task was to prepare indenone **3.1**. Several approaches were investigated.[6] One of these was based on a Nazarov cyclization,[9] and it was this route that was eventually used for the work described in this Thesis. Sannigrahi developed this approach first in a simple model system (Scheme 4). Aldehyde **4.1** was treated with isopropenylmagnesium bromide, and the resulting alcohol was oxidized to ketone **4.3**. When this was stored in concentrated H_2SO_4 for 6 h, it was converted by Nazarov cyclization[9] into


OMe
OHC
OMe
4.1
MgBr
1.5 equiv.
Et$_2$O,
60%
OH
OMe
OMe
4.2
PCC,
CH$_2$C$_2$, 95%
O
OMe
OMe
4.3
H$_2$SO$_4$,
6 h, 25 °C,
65%
O
OMe
OMe
4.4
O
OMe
OMe
4.5

Scheme 4

indanone **4.4**. As this was a model sequence, no attempt was made to desaturate the indanone (**4.4** → **4.5**); this should, in any case, be a very simple operation. With a method for making the five-membered ring available, attention was turned to the preparation of an aldehyde corresponding to **4.1**, but carrying suitable substituents for elaboration into the methyl and hydroxyethyl groups of puraquinonic acid. To this end, the ester **5.1**[10] was elaborated as shown in Schemes 5 and 6. These reactions represent a refined version of several related sequences studied by Sannigrahi.

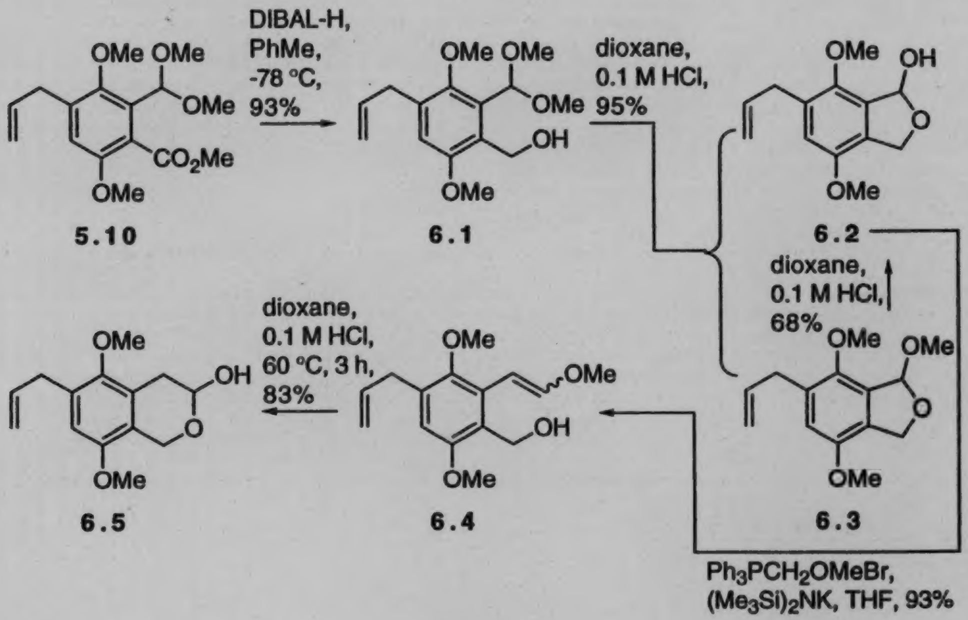
A classical Sandmeyer reaction[11] served to convert **5.1** into the bromide **5.2**, and this, in turn, was converted into


NaNO$_2$, HBr, water, CuBr, 100 °C, 81%
DIBAL-H, PhMe, -78 °C to room temp, 99%
PCC, CH$_2$Cl$_2$, 95%
BCl$_3$, CH$_2$Cl$_2$, -78 °C to room temp, 96%
allyl bromide, NaH, DMF, 0 °C to room temp, 83%
degassed *trans*-decalin, 200 °C, 74%
MeI, K$_2$CO$_3$, DMF, 70 °C, 97%
HC(OMe)$_3$, TsOH.H$_2$O, MeOH, 70 °C, 96%
BuLi, THF, -78 °C, MeOC(O)CN, 88%
5.1
5.2
5.3
5.4
5.5
5.6
5.7
5.8
5.9
5.10

Scheme 5

aldehyde **5.4** by reduction to the alcohol (**5.2** → **5.3**) and oxidation.[12] Regioselective demethylation, directed by the aldehyde group (**5.4** → **5.5**),[13] allylation (**5.5** → **5.6**),[14] and thermal Claisen rearrangement (**5.6** → **5.7**) then gave phenol **5.7**. This was methylated in the standard way, and protected as its dimethyl acetal **5.9**. The use of a *dimethyl* instead of a *cyclic* acetal and the indicated order of the last four steps – allylation, rearrangement, *O*-methylation and acetalization – was the best of several possibilities that were examined. At this point, halogen-metal exchange, and treatment with Mander's reagent[15] gave ester **5.10**. Since the ester is destined to provide the methyl substituent of puraquinonic acid, it would seem more logical to quench the anion derived from **5.9** with MeI rather than with Mander's reagent, but this approach eventually incurred complications, as described below (see discussion associated with Scheme 12).

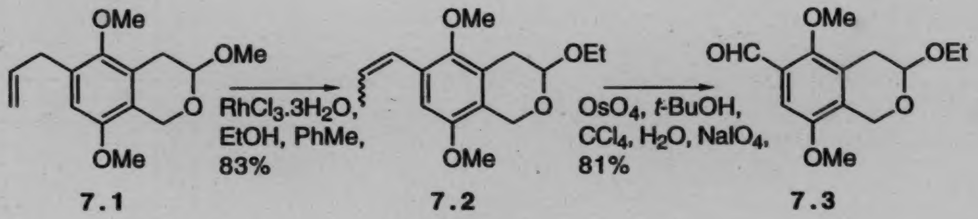
The acetal and ester groups were next modified, as shown in Scheme 6. Ester reduction gave alcohol **6.1**, and acid hydrolysis then afforded a mixture of lactol **6.2** and the


DIBAL-H, PhMe, -78 °C, 93%
dioxane, 0.1 M HCl, 95%
dioxane, 0.1 M HCl, 68%
dioxane, 0.1 M HCl, 60 °C, 3 h, 83%
Ph3PCH2OMeBr, (Me3Si)2NK, THF, 93%
OMe
OH
CO2Me
5.10
6.1
6.2
6.3
6.4
6.5

Scheme 6

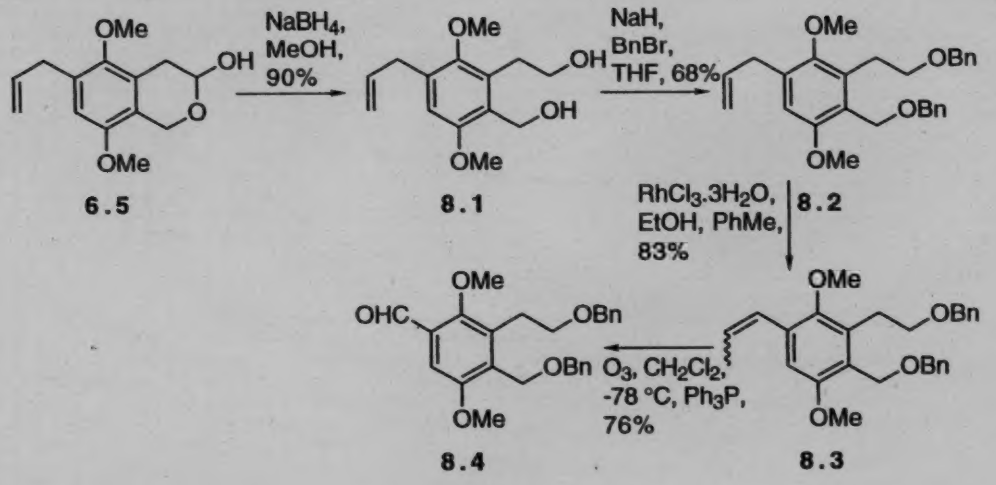
corresponding lactol methyl ether **6.3**. The latter could be hydrolyzed to the lactol by further treatment with acid. The second carbon of the eventual hydroxyethyl side chain of puraquinonic acid was then introduced by Wittig olefination[16] of the lactol to obtain a mixture of *Z* and *E* enol ethers **6.4**. Mild acidic hydrolysis then gave lactol **6.5** (83%) together with a small amount of the corresponding lactol methyl ether.

Lactol **6.5** and its methyl ether were elaborated in several ways. In the first series of reactions the lactol methyl ether **7.1** (obtained in early experiments as a byproduct in the formation of **6.5**) was treated with $RhCl_3.3H_2O$[17] in order to shift the pendant double bond into conjugation with the aromatic ring (Scheme 7), and the double bond was then cleaved, using the classical OsO_4-$NaIO_4$ combination. During the double bond isomerization alkoxy exchange occurred so that the product (**7.2**) was a lactol ethyl ether.



Scheme 7

In the second series of experiments (Scheme 8), lactol **6.5** was reduced to diol **8.1**, and the hydroxyls were protected by benzylation. The pendant double bond was moved,

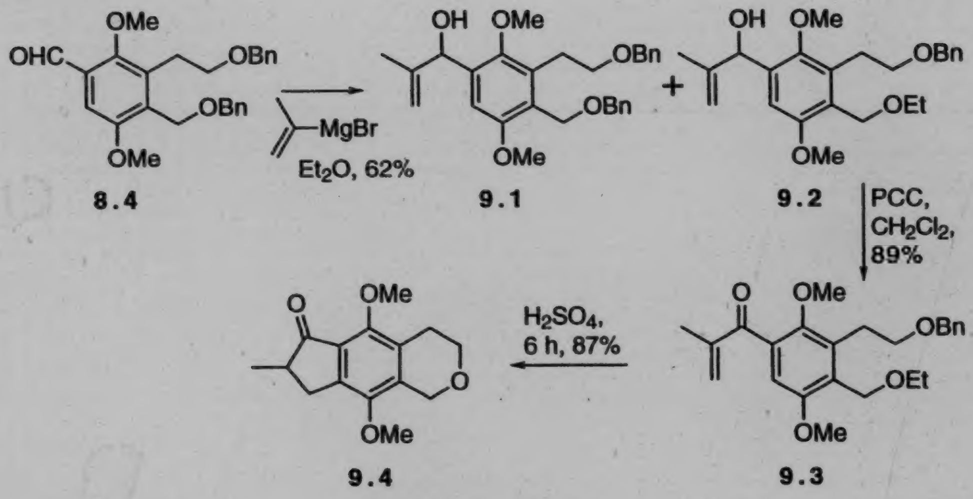


Scheme 8

as before,[17] and then cleaved by ozonolysis.

Aldehyde **8.4** served as the key intermediate in a method for building the five-membered ring. Condensation with isopropenylmagnesium bromide gave alcohols **9.1** and **9.2** (Scheme 9). The fact that one of the benzyl groups had been replaced by an ethoxy group made no difference to the overall scheme. Oxidation of **9.2** set the stage for a Nazarov cyclization[9] (**9.3** → **9.4**), which was accomplished in 87% yield by storing **9.3** in concentrated H_2SO_4 for 6 h. During the Nazarov cyclization the two protected side chains were incorporated into a ring, but it was felt – in the event, correctly (see later) – that this structural feature could be modified so as to generate the required methyl and hydroxyethyl substituents.

The experiments summarized in Scheme 9 represent the most advanced stages of Sannigrahi's work, and I took over at that point.

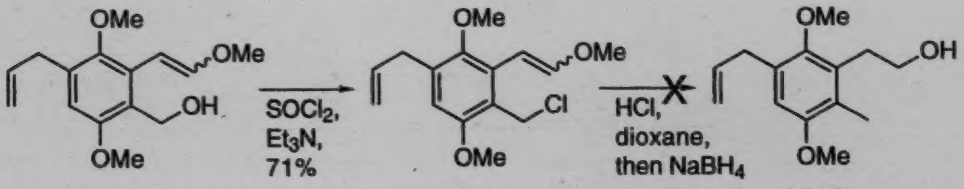

OHC
OMe
OBn
OBn
OMe
8.4
MgBr
Et₂O, 62%
OH
OMe
OBn
OBn
OMe
9.1
+
OH
OMe
OBn
OEt
OMe
9.2
PCC,
CH₂Cl₂,
89%
O
OMe
OBn
OEt
OMe
9.3
H₂SO₄,
6 h, 87%
O
OMe
O
OMe
9.4

Scheme 9

DISCUSSION OF RESEARCH RESULTS

Synthesis of (±)-puraquinonic acid

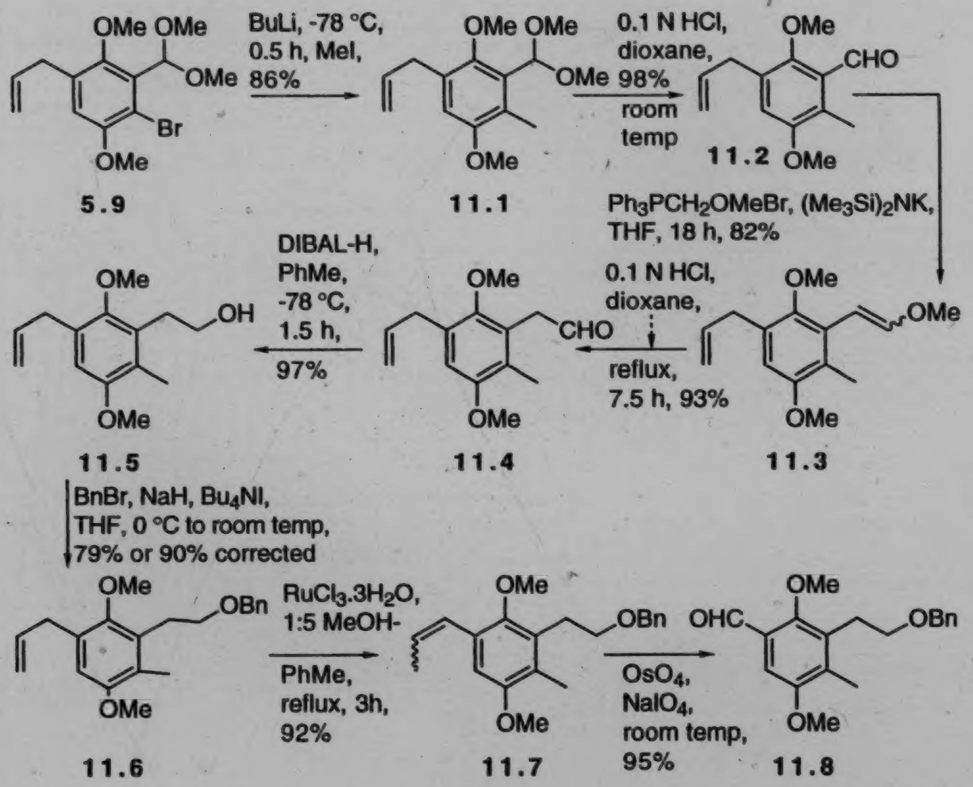
We first decided to introduce the aromatic methyl substituent of puraquinonic acid at an early stage. To this end, the enol ethers **6.4** were converted (Scheme 10) into the corresponding benzylic chlorides **10.1**, in the expectation that acid hydrolysis (to release an aldehyde group), and treatment with $NaBH_4$ would serve to generate **10.2**. However, the hydrolysis-reduction step that ought to have yielded **10.2**, gave, instead, a complex mixture. We turned, therefore, to an earlier intermediate (**5.9**).



6.4 **10.1** **10.2**

Scheme 10

Halogen-metal exchange and reaction with MeI, followed by acid hydrolysis (Scheme 11, **5.9** → **11.1** → **11.2**) proceeded without incident, and the aldehyde group was then subjected to Wittig olefination, as before (cf. Scheme 6). Acid hydrolysis released an aldehyde group (Scheme 11, **11.3** → **11.4**). Reduction with DIBAL-H and benzylation – all under standard conditions – took the route as far as **11.6**. The double bond was then isomerized[17] and cleaved (**11.6** → **11.7**

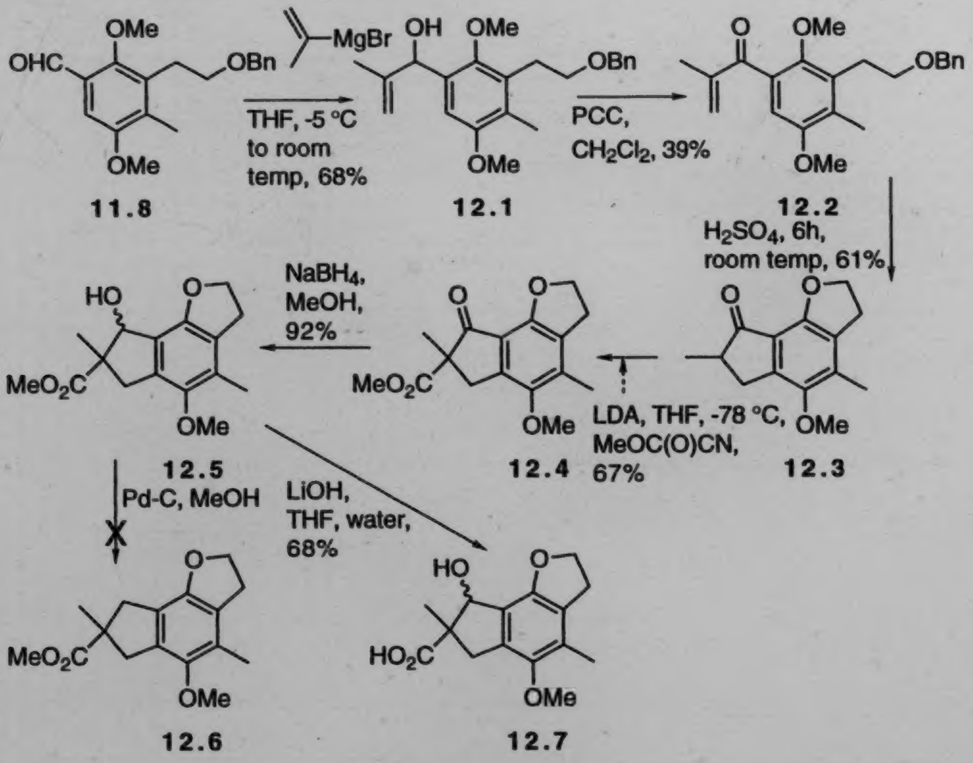

OMe OMe
OMe
Br
OMe
5.9
BuLi, -78 °C, 0.5 h, MeI, 86%
11.1
0.1 N HCl, dioxane, 98% room temp
CHO
11.2
Ph3PCH2OMeBr, (Me3Si)2NK, THF, 18 h, 82%
11.3
0.1 N HCl, dioxane, reflux, 7.5 h, 93%
11.4
DIBAL-H, PhMe, -78 °C, 1.5 h, 97%
OH
11.5
BnBr, NaH, Bu4NI, THF, 0 °C to room temp, 79% or 90% corrected
OBn
11.6
RuCl3.3H2O, 1:5 MeOH-PhMe, reflux, 3h, 92%
11.7
OsO4, NaIO4, room temp, 95%
OHC
11.8

Scheme 11

→ **11.8**), bringing us to the point where assembly of the five-membered ring could be started.

Aldehyde **11.8** reacted (Scheme 12) with isopropenylmagnesium bromide to give **12.1** (68%) and a very small amount (2% yield) of the corresponding phenol in which the methoxy group adjacent to the hydroxyl had been demethylated. PCC oxidation of **12.1** led to ketone **12.2**, and this was stored in concentrated H_2SO_4 to effect Nazarov cyclization.[9] The five-membered ring did indeed form, but at the same time the oxygenated side chain and adjacent methoxy group were unexpectedly converted into a heterocycle (**12.2** → **12.3**).

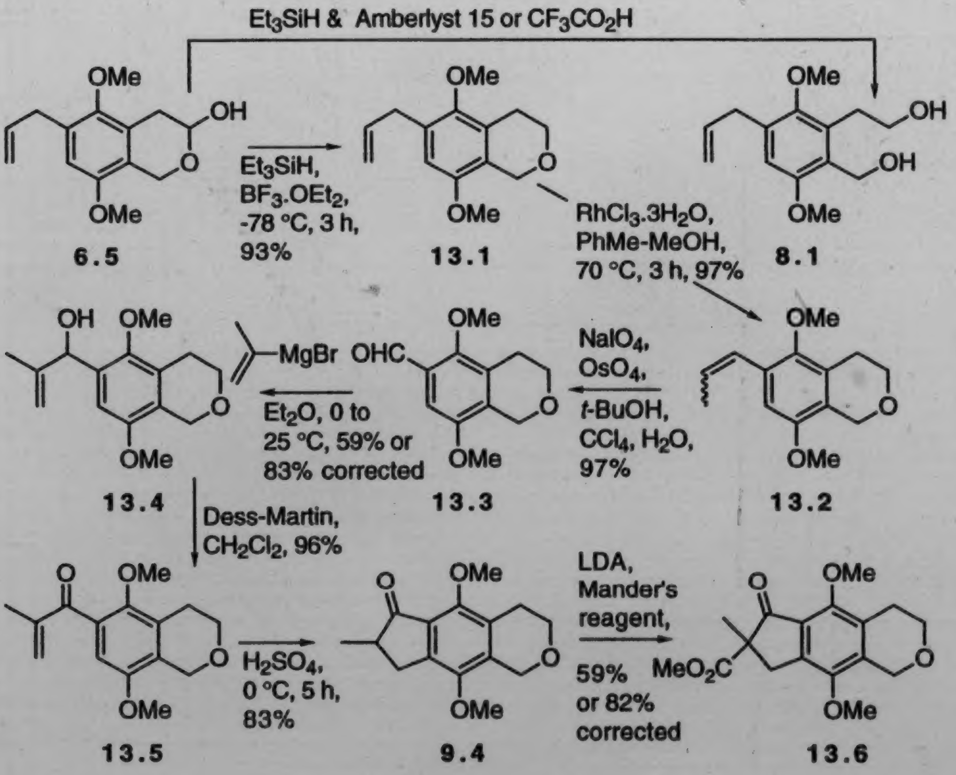
At the time, this was not regarded as significant, as we felt that compound **12.3** could be accommodated into our plans without any additional steps.


11.8
THF, -5 °C to room temp, 68%
12.1
PCC, CH2Cl2, 39%
12.2
H2SO4, 6h, room temp, 61%
12.3
LDA, THF, -78 °C, MeOC(O)CN, 67%
12.4
NaBH4, MeOH, 92%
12.5
Pd-C, MeOH
12.6
LiOH, THF, water, 68%
12.7

Scheme 12

Acylation of **12.3** with Mander's reagent[15] produced (67%) the desired β-keto ester **12.4**, and we proceeded to try to remove the unwanted ketone oxygen by reduction ($NaBH_4$, MeOH, 92%) and hydrogenolysis. Surprisingly, the hydrogenolysis step (**12.5** → **12.6**) did not work. Hydrolysis of **12.5** gave the acid **12.7**. When we treated **12.7** with BBr_3 an unidentified brown solid was obtained, and we could not detect (^1H NMR) any product in which the heterocyclic ring had been opened. In retrospect, we realize that we should have tried $(NH_4)_2Ce(NO_3)_6$ in the presence of 2,6-pyridinedicarboxylic acid *N*-oxide (see later) in order to open the heterocycle.[18]

Because of our failure to open the five-membered heterocyclic ring of the dihydrobenzofuran **12.7**, we decided to return to the isochroman series. Formation of isochroman **9.4** had been unexpected, but we realized that we might benefit from the process, because it represents a method for protection of the hydroxyethyl side chain. In fact, formation of the heterocyclic ring in **9.4** turned out to be a key factor in the success of our approach to puraquinonic


Et3SiH & Amberlyst 15 or CF3CO2H
OMe
OH
O
OMe
6.5
Et3SiH,
BF3.OEt2,
-78 °C, 3 h,
93%
13.1
RhCl3.3H2O,
PhMe-MeOH,
70 °C, 3 h, 97%
8.1
OH
MgBr
Et2O, 0 to
25 °C, 59% or
83% corrected
OHC
13.4
13.3
NaIO4,
OsO4,
t-BuOH,
CCl4, H2O,
97%
13.2
Dess-Martin,
CH2Cl2, 96%
H2SO4,
0 °C, 5 h,
83%
13.5
9.4
LDA,
Mander's
reagent,
59%
or 82%
corrected
MeO2C
13.6

Scheme 13

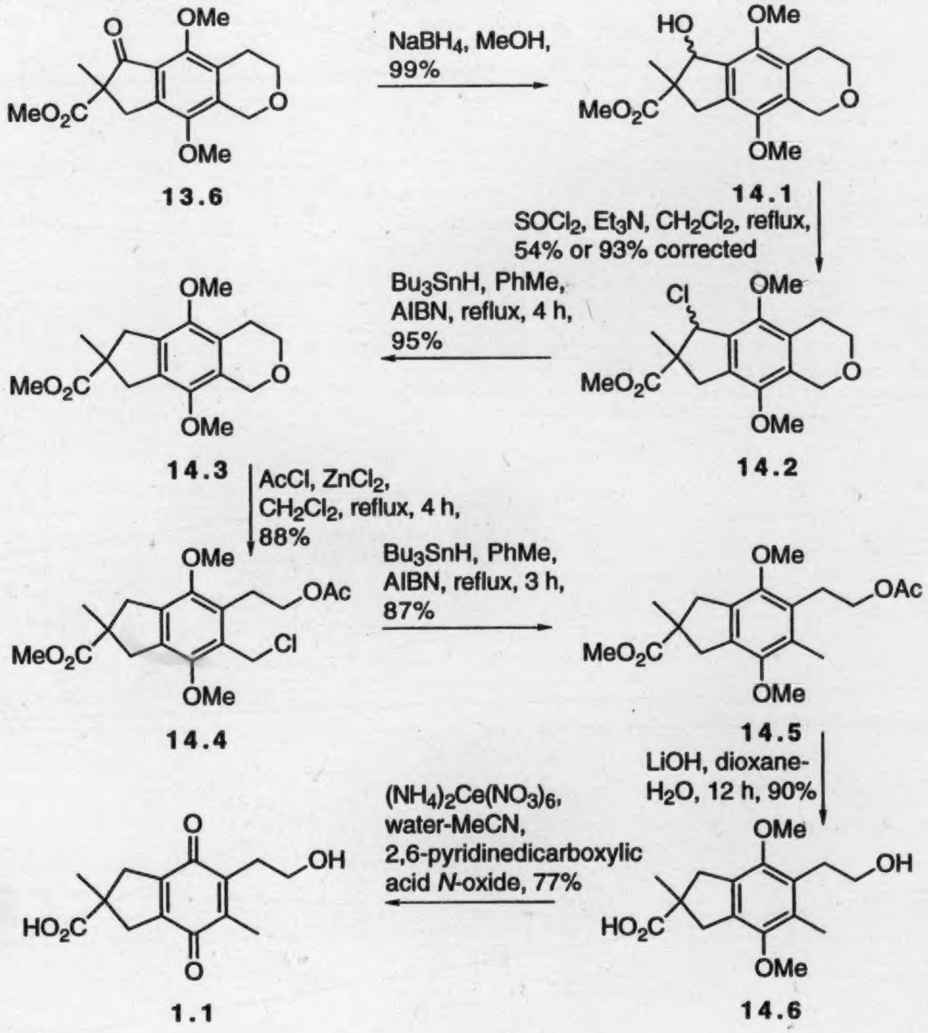
acid.

We decided to generate the isochroman at an earlier stage, and to this end, lactol **6.5** was elaborated as shown in Scheme 13.

Treatment of **6.5** with Et_3SiH in the presence of *freshly* distilled $BF_3.OEt_2$ gave **13.1**.[19] A very high yield (93%) was obtained, but only if freshly distilled $BF_3.OEt_2$ was used. Use of Et_3SiH together with Amberlyst 15 gave mainly diol **8.1**, which was inert to further treatment with Amberlyst 15 or CF_3CO_2H. When diol **8.1** was treated with Et_3SiH and CF_3CO_2H the hydroxymethyl group was not converted into a methyl group.

The next steps followed the sequence established in earlier work. Migration of the pendant double bond,[17] and oxidative double bond cleavage (**13.1** → **13.2** → **13.3**) set the stage for elaboration of the five-membered ring. Reaction with isopropenylmagnesium bromide — an excess must be avoided, in order to suppress *O*-demethylation — and Dess-Martin oxidation gave the substrate (**13.5**) required for the Nazarov cyclization. This occurred without incident; in particular, the presence of the oxygen heterocycle precluded unwanted involvement of one of the methoxy groups. The Nazarov cyclization product (**9.4**) was then acylated with Mander's reagent. All the steps of Scheme 13 worked well, although in two cases correction had to be made for recovered starting material.

At this point, it was necessary to deoxygenate the ketone and open the heterocyclic ring. We had planned to effect both steps by sequential hydride reduction and hydrogenolysis, there being some precedent^{20} for opening the oxygen heterocycle. However, this approach was unsuccessful,


13.6
NaBH4, MeOH, 99%
14.1
SOCl2, Et3N, CH2Cl2, reflux, 54% or 93% corrected
14.2
Bu3SnH, PhMe, AIBN, reflux, 4 h, 95%
14.3
AcCl, ZnCl2, CH2Cl2, reflux, 4 h, 88%
14.4
Bu3SnH, PhMe, AIBN, reflux, 3 h, 87%
14.5
LiOH, dioxane-H2O, 12 h, 90%
14.6
(NH4)2Ce(NO3)6, water-MeCN, 2,6-pyridinedicarboxylic acid N-oxide, 77%
1.1
OMe
MeO2C
HO2C
OAc
OH
Cl
HO

Scheme 14

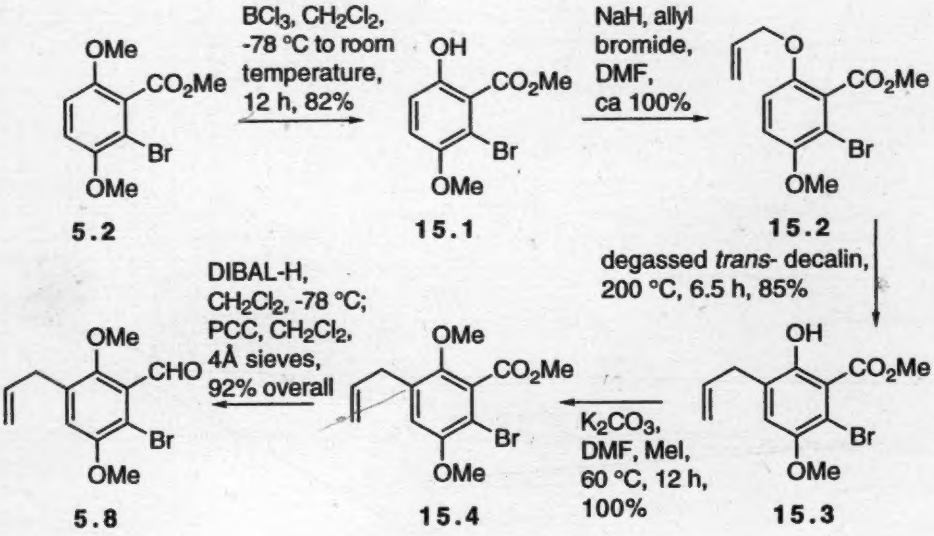
and the tasks were accomplished as follows. Reduction of ketone **13.6** with $NaBH_4$ gave alcohols **14.1**, and these were treated with $SOCl_2$ in order to replace the OH by Cl (Scheme 14, **13.6** → **14.1** → **14.2**). Stannane reduction then completed the deoxygenation process.

Next, the heterocycle was opened by a method[21] that involves heating with AcCl in the presence of $ZnCl_2$. This operation generated the acetoxy chloride **14.4**, from which the halogen was removed by stannane reduction (**14.3** → **14.4** → **14.5**). Base hydrolysis with LiOH removed the acetyl group and hydrolyzed the methyl ester, releasing hydroxy acid **14.6**. Finally, oxidation with $(NH_4)_2Ce(NO_3)_6$ in the presence of 2,6-pyridinedicarboxylic acid *N*-oxide[18] generated the quinone system, giving (±)-puraquinonic acid in 77% yield.[22]

Generation of the quinone as just described was the result of several exploratory experiments. Initially, we had treated **14.5** with $(NH_4)_2Ce(NO_3)_6$ but recovered the dimethoxy compound unchanged. Examination of the literature revealed the beneficial effects of adding 2,6-pyridinedicarboxylic acid *N*-oxide,[18] and **14.5** was then converted into the corresponding quinone (77%) by using the additive. However, attempts to hydrolyze the acetate and methyl ester groups of the resulting quinone resulted in destruction of our compound. For this reason, the hydrolysis was done before treatment with $(NH_4)_2Ce(NO_3)_6$.

During the course of our experiments we used up the supply of starting material (**5.10**) left by Sannigrahi and, in preparing more of its precursor **5.8**, we followed a slightly different route (see Scheme 15) from the one she had used.

Ester **5.2** was selectively demethylated with BCl_3 – use of BBr_3 had been found by Sannigrahi to cause extensive demethylation of both ether groups – and the resulting phenol **15.1** was allylated in the usual way. Thermal Claisen rearrangement and remethylation gave ester **15.4**, which was reduced down to the alcohol level, and reoxidized to aldehyde **5.8**. This route is one step shorter than the previous method, but was not used routinely since the first run provided sufficient material to complete our work. (As we did not repeat the sequence to obtain good spectral data, we have not included the details in the Experimental Section; our sample of **5.8** was identical to the previous samples.)

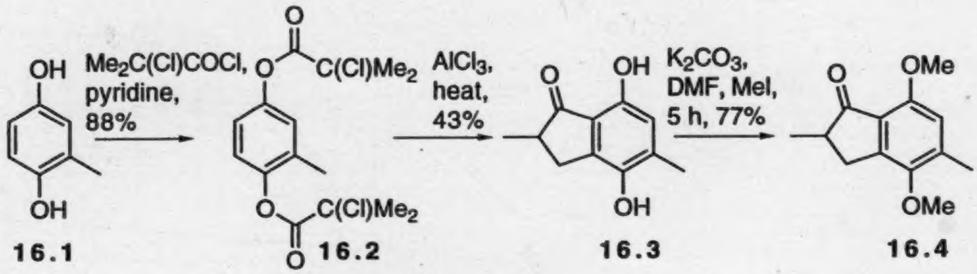

OMe
CO2Me
Br
OMe
5.2
BCl3, CH2Cl2, -78 °C to room temperature, 12 h, 82%
OH
CO2Me
Br
OMe
15.1
NaH, allyl bromide, DMF, ca 100%
O
CO2Me
Br
OMe
15.2
degassed trans- decalin, 200 °C, 6.5 h, 85%
OH
CO2Me
Br
OMe
15.3
K2CO3, DMF, MeI, 60 °C, 12 h, 100%
OMe
CO2Me
Br
OMe
15.4
DIBAL-H, CH2Cl2, -78 °C; PCC, CH2Cl2, 4Å sieves, 92% overall
OMe
CHO
Br
OMe
5.8

Scheme 15

Second route to (±)-puraquinonic acid

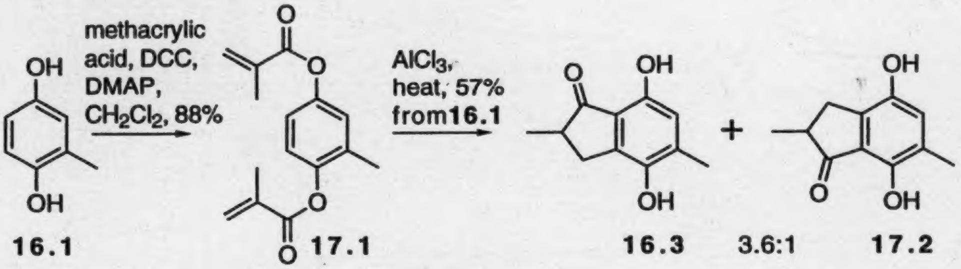
Having completed the above synthesis, we embarked on a shorter route that also bypasses the requirement that the hydroxyethyl side chain be protected in such a way that it cannot close onto the oxygen of the adjacent methoxy group during the Nazarov cyclization.

The bisphenol **16.1** was acylated with $Me_2C(Cl)COCl$ and the product (**16.2**) was treated with $AlCl_3$, first at room temperature, and then at 190 °C, both steps being taken from the patent literature.[23] No yield is given in the patent for the first step, but, not surprisingly, it worked well (88%).


OH
OH
Me₂C(Cl)COCl, pyridine, 88%
C(Cl)Me₂
C(Cl)Me₂
AlCl₃, heat, 43%
K₂CO₃, DMF, MeI, 5 h, 77%
OMe
OMe
16.1
16.2
16.3
16.4

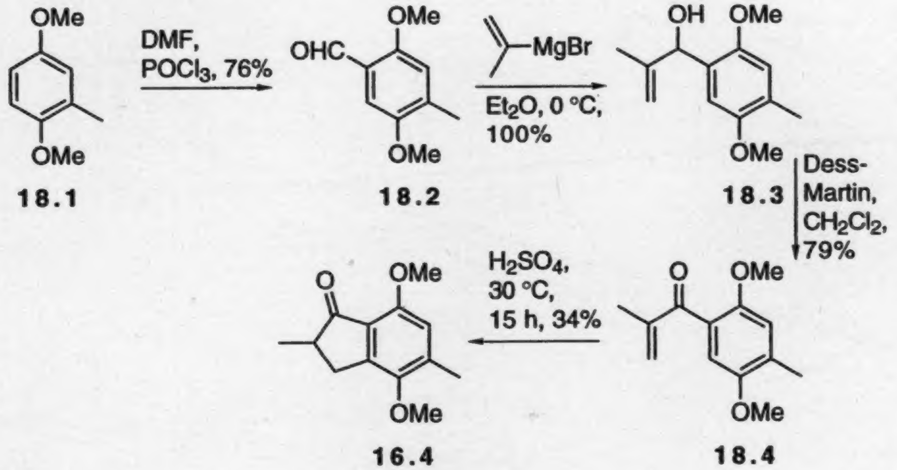
Scheme 16

The $AlCl_3$-induced Fries rearrangement and Nazarov cyclization (**16.2** → **16.3**) (for which, again, no yield is given) afforded the indanone **16.3** in 43% yield. Although not efficient, the reaction is easy to do and gram quantities of **16.3** and **16.4** are readily available.


OH
OH
methacrylic acid, DCC, DMAP, CH₂Cl₂, 88%
AlCl₃, heat, 57% from16.1
+
16.1
17.1
16.3
3.6:1
17.2

Scheme 17

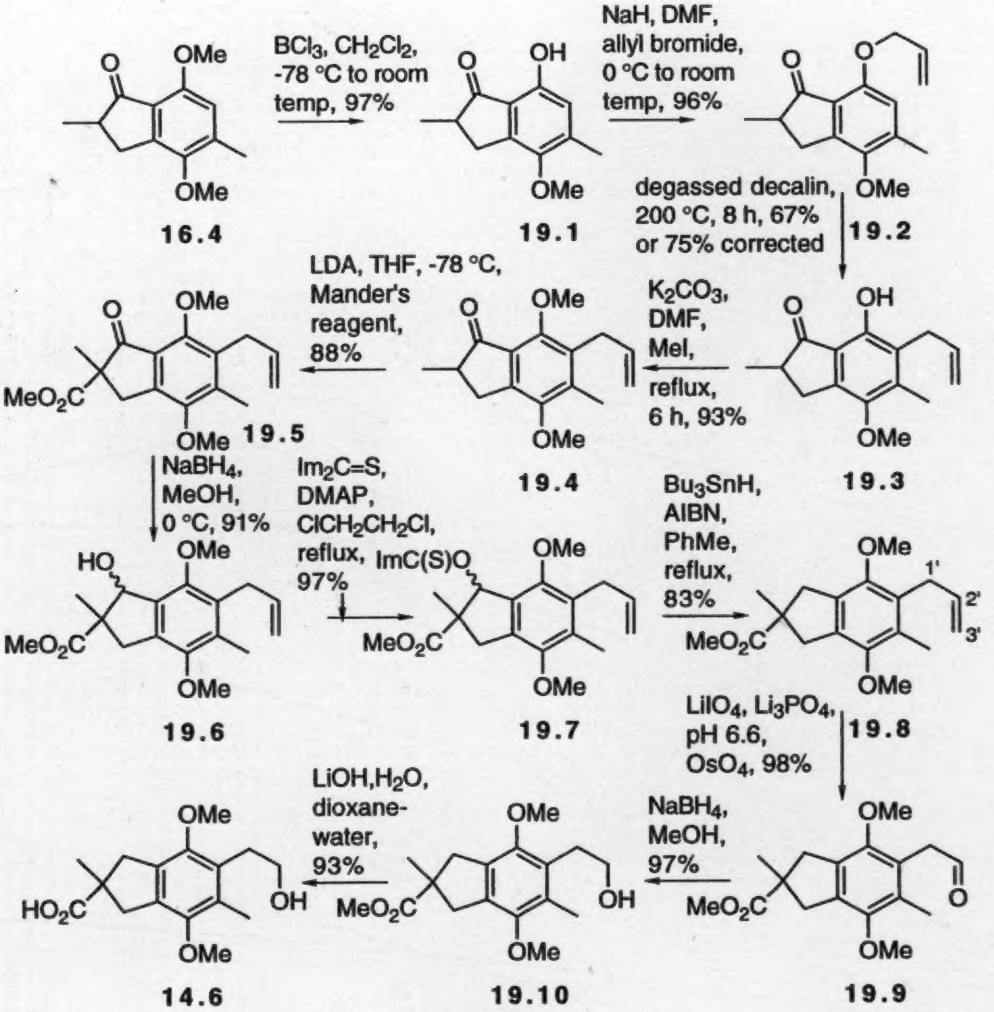
An alternative route was also examined, in which the need to prepare $Me_2C(Cl)COCl$ is avoided and commercial methacrylic acid is used instead, as shown in Scheme 17. Methylation of the mixture of **16.3** and **17.2** allowed easy separation of the isomers and afforded the bis-methyl ether **16.4**. This substance was also prepared in yet a different way (Scheme 18).


OMe
OMe
18.1
DMF,
POCl3, 76%
OHC
OMe
OMe
18.2
MgBr
Et2O, 0 °C,
100%
OH
OMe
OMe
18.3
Dess-
Martin,
CH2Cl2,
79%
O
OMe
OMe
18.4
H2SO4,
30 °C,
15 h, 34%
O
OMe
OMe
16.4

Scheme 18

Formylation[24] of the commercially available bis-ether **18.1** gave **18.2** in 76% yield. Reaction with isopropenylmagnesium bromide and Dess-Martin oxidation[25] both worked well (100% and 79%, respectively) but, when the resulting enone (**18.4**) was subjected to our standard conditions for Nazarov cyclization, the desired product was formed in poor yield (34%). Use of $Me_3SiOSO_2CF_3$ destroyed the starting material (**18.4**) without giving any of the required product; **18.4** was inert to $TiCl_4$. Consequently, this route was abandoned.

With **16.4** in hand, we proceeded as summarized in Scheme 19.


BCl3, CH2Cl2, -78 °C to room temp, 97%
NaH, DMF, allyl bromide, 0 °C to room temp, 96%
degassed decalin, 200 °C, 8 h, 67% or 75% corrected
K2CO3, DMF, MeI, reflux, 6 h, 93%
LDA, THF, -78 °C, Mander's reagent, 88%
NaBH4, MeOH, 0 °C, 91%
Im2C=S, DMAP, ClCH2CH2Cl, reflux, 97%
Bu3SnH, AIBN, PhMe, reflux, 83%
LiIO4, Li3PO4, pH 6.6, OsO4, 98%
NaBH4, MeOH, 97%
LiOH,H2O, dioxane-water, 93%
16.4
19.1
19.2
19.3
19.4
19.5
19.6
19.7
19.8
19.9
19.10
14.6
1'
2'
3'

Scheme 19

Selective demethylation of **16.4**, directed by the carbonyl group, was achieved on treatment with BCl_3 (-78 °C to room temperature, 97%).[13a] From that point, *O*-allylation (**19.1** → **19.2**, 96% or 99% after correction for recovered **19.1**), Claisen rearrangement (**19.2** → **19.3**, 200 °C, 8 h, 67%, or 75% after correction for recovered **19.2**), and *O*-methylation (93%) gave the highly substituted indanone derivative **19.4**. Acylation with Mander's reagent [LDA, MeOC(O)CN, 88%] then provided keto ester **19.5**, which contains all the required skeletal carbons and the appropriate functionality for conversion into puraquinonic acid.

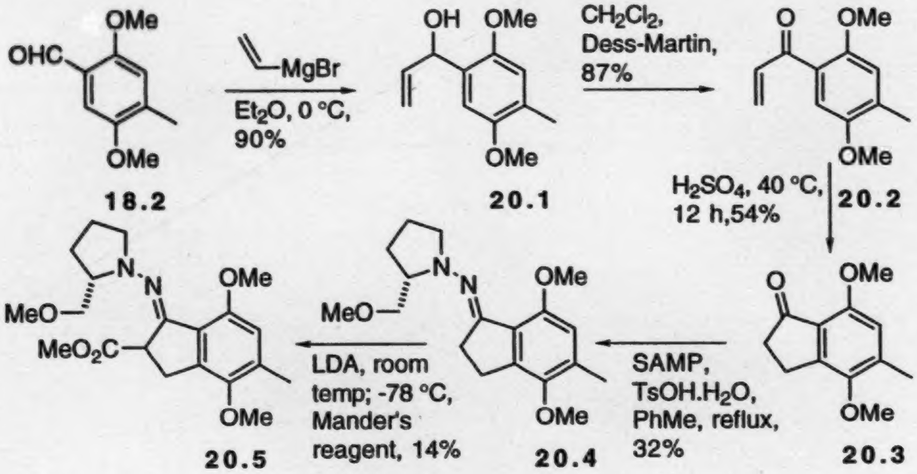
An attempt to remove the ketonic oxygen by Wolff-Kishner reduction was not successful, the ketone being destroyed. Reduction of the ketone carbonyl ($NaBH_4$, MeOH, 91%) gave alcohols **19.6**, which could be converted ($SOCl_2$, Et_3N) in low yield (23%) into the corresponding unstable chlorides (not shown in Scheme 19). However, radical deoxygenation by the Barton method (**19.6** → **19.7** → **19.8**, 80% overall) took the route to a stage where the 2′,3′ double bond had to be cleaved. This seemingly straightforward operation was initially troublesome, as ozonolysis resulted in destruction of the starting material, and treatment with OsO_4-$NaIO_4$ under standard conditions[26] gave the required aldehyde **19.9** only in low yield (ca 22%). However, use of OsO_4-$LiIO_4$ in an aqueous phosphate buffer at pH 6.6[27] afforded **19.9** in 98% yield, and $NaBH_4$ reduction (97%) led to alcohol **19.10**. Simple hydrolysis (LiOH, aqueous THF, 93%) liberated the parent acid **14.6**,[22,28] which we had previously[22] oxidized [$(NH_4)_2Ce(NO_3)_6$, 2,6-pyridinedicarboxylic acid *N*-oxide,[18] 77%] to racemic puraquinonic acid.

In the present route, formation of the 5-membered ring at an early stage avoids complications engendered by the presence of the (2′-oxyethyl) side chain, and a considerable shortening of the synthesis results. Our attempts to effect oxidative cleavage of the allyl side chain of **19.8** illustrate the significant improvement that can be achieved in the Lemieux-Johnson oxidation by controlling the pH. This method deserves to be better known.

Studies on the synthesis of optically pure puraquinonic acid

(a) Attempts to use SAMP derivatives

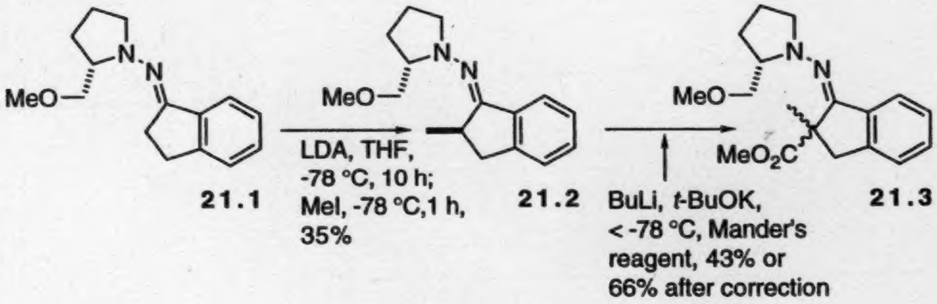
Our first approach was based on the SAMP derivative[29] of indanone **20.3**, which was prepared from **18.2**, as shown in Scheme 20, the experiments being of exactly the same type as those used previously. Our choice of **20.3** was forced on us because we were unable to make the SAMP derivative of **16.4**, presumably because the carbonyl is too hindered. The SAMP


OHC
OMe
OMe
18.2
MgBr
Et2O, 0 °C, 90%
OH
OMe
OMe
20.1
CH2Cl2, Dess-Martin, 87%
O
OMe
OMe
20.2
H2SO4, 40 °C, 12 h,54%
O
OMe
OMe
20.3
SAMP, TsOH.H2O, PhMe, reflux, 32%
N
N
MeO
OMe
OMe
20.4
LDA, room temp; -78 °C, Mander's reagent, 14%
N
N
MeO
MeO2C
OMe
OMe
20.5

Scheme 20

derivative of **20.3** was obtained in only 32% yield. The inefficiency of the process is due to steric or electronic factors generated by the *peri* MeO group, since indanone itself gave the SAMP derivative in 94% yield under the same conditions. When **20.4** was deprotonated with LDA in THF at -78 °C for 40 min and treated with Mander's reagent or with D_2O, we observed no acylation or deuteration products. However, when the LDA-**20.4** mixture was warmed to room temperature and kept at room temperature for 3 h before addition of Mander's reagent at -78 °C, the desired acylation product was indeed formed – but only in 14% yield and as a mixture of isomers. In retrospect, we should have added a second equivalent of LDA (since the desired product is more acidic than the initial anion, and must have been deprotonated by it); in that case, protonation on workup may have occurred in an asymmetric fashion.

We decided to examine the simpler SAMP hydrazone **21.1** (Scheme 21), which was easily made from indanone. Standard methylation gave a single isomer, which we assume to have the

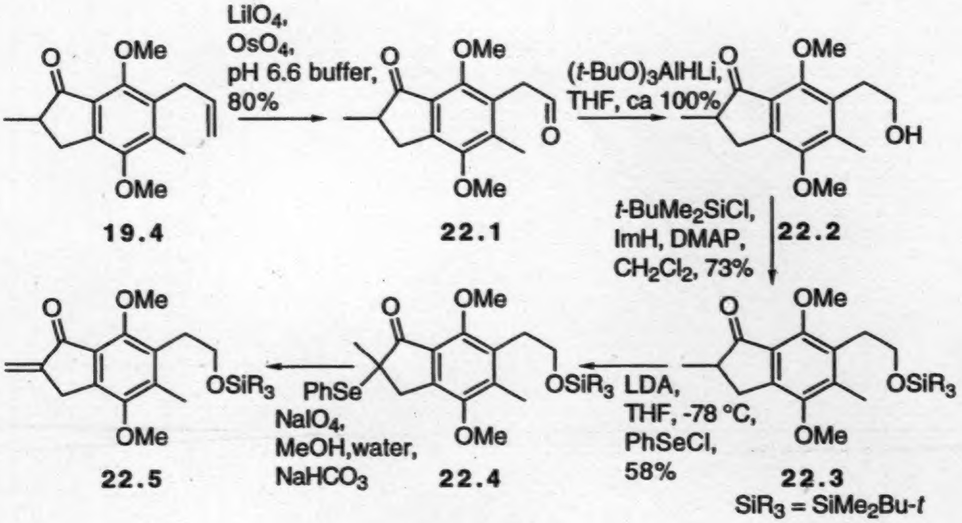


Scheme 21

stereochemistry shown. Deprotonation and acylation with Mander's reagent gave **21.3** in 43% yield (or 66% after correction for recovered **21.2**). Unfortunately, the material was a 1:1 mixture of diastereoisomers; we conclude that the SAMP derivatives are unsuitable for our purpose, and we turned our attention to the original plan summarized in Scheme 3.

(b) Approach based on an optically pure allylic alcohol

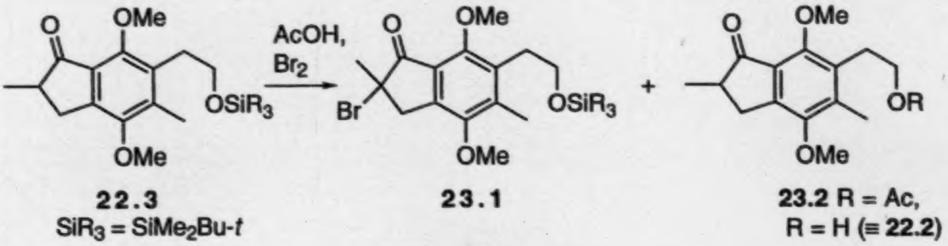
In order to implement the original plan (see Scheme 3), the pendant double bond in ketone **19.4** was first cleaved (Scheme 22), using $LiIO_4$-OsO_4. With this reagent combination[27] the yield was 80%, while under the standard Lemieux-Johnson conditions the yield was 64%. The resulting aldehyde group was selectively reduced to an alcohol, which



O
OMe
OMe
19.4
LiIO4,
OsO4,
pH 6.6 buffer,
80%
22.1
(t-BuO)3AlHLi,
THF, ca 100%
OH
22.2
t-BuMe2SiCl,
ImH, DMAP,
CH2Cl2, 73%
OSiR3
22.3
SiR3 = SiMe2Bu-t
LDA,
THF, -78 °C,
PhSeCl,
58%
PhSe
22.4
NaIO4,
MeOH,water,
NaHCO3
22.5

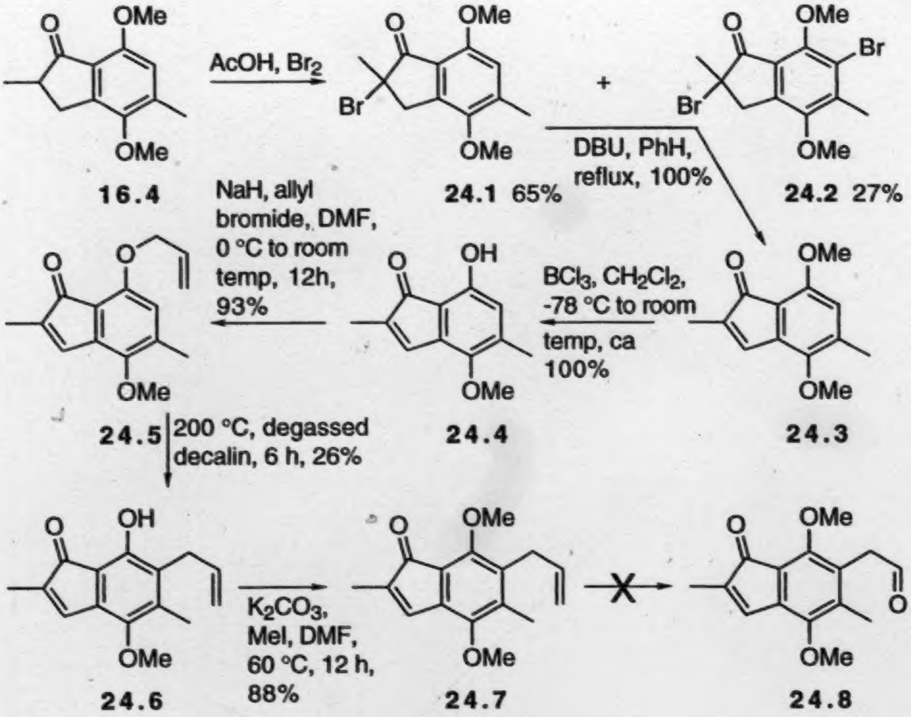
Scheme 22

was then protected by silylation (**19.4** → **22.1** → **22.2** → **22.3**). Phenylselenation in the usual way gave keto selenide **22.4**, but when this was treated with $NaIO_4$ it was recovered largely unchanged together with a trace of the exocyclic olefin **22.5**. We decided, therefore, to introduce the required double bond by bromination-dehydrobromination, and so ketone **22.3** was treated with Br_2 in AcOH (Scheme 23). Some of the desired **23.1** was obtained, but there was extensive desilylation and acylation (**23.1**:**23.2**:**22.2** = 1:3.8:3 by ^1H NMR). To avoid these side reactions, we decided to perform the bromination-dehydrobromination before elaborating the oxyethyl sidechain.



Scheme 23

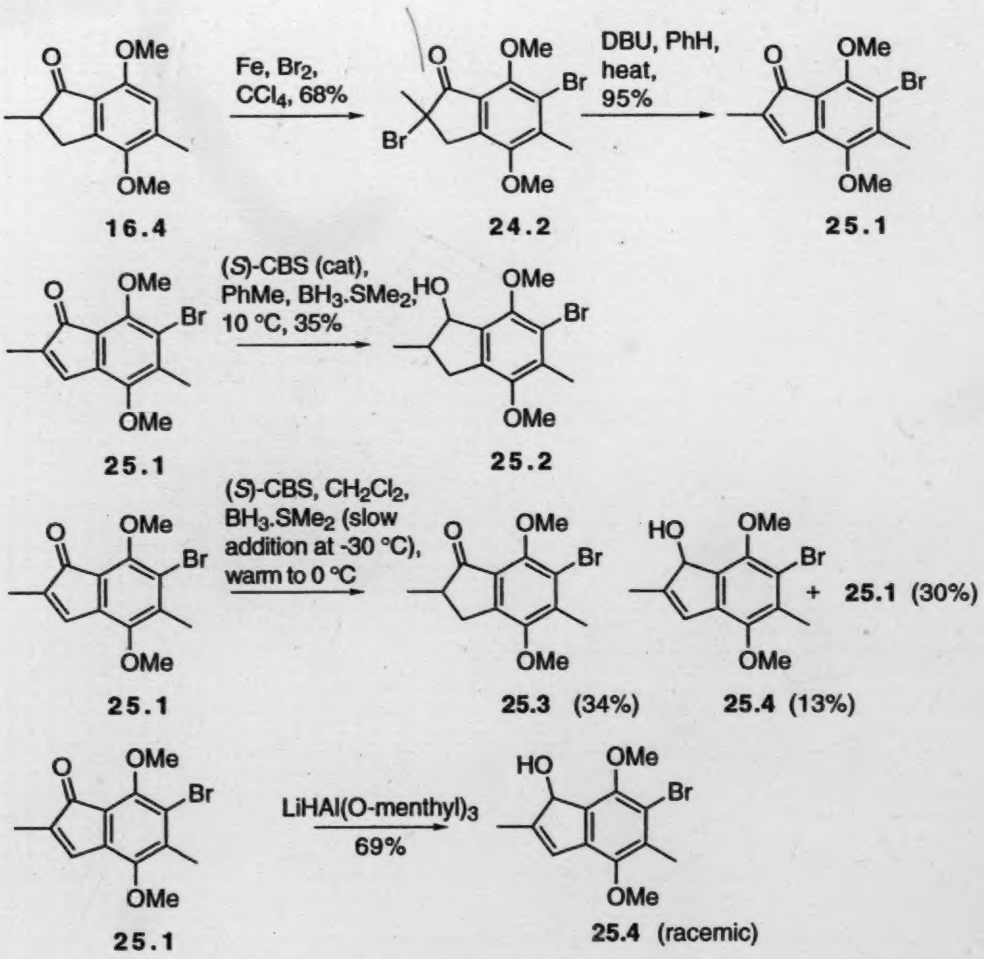
Bromination of **16.4** gave mainly the monobromide **24.1** (Scheme 24, 65%), but an appreciable amount (25%) of the dibromide **24.2** was also formed. When **24.1** was heated with DBU in PhH, it was smoothly converted into the desired enone **24.3**. This was selectively demethylated (BCl_3, ca 100%), and subjected to our usual sequence of allylation, Claisen rearrangement, and remethylation, bringing the work to the stage of compound **24.7**. When we attempted to cleave the


AcOH, Br$_2$
DBU, PhH, reflux, 100%
NaH, allyl bromide, DMF, 0 °C to room temp, 12h, 93%
BCl$_3$, CH$_2$Cl$_2$, -78 °C to room temp, ca 100%
200 °C, degassed decalin, 6 h, 26%
K$_2$CO$_3$, MeI, DMF, 60 °C, 12 h, 88%
16.4
24.1 65%
24.2 27%
24.3
24.4
24.5
24.6
24.7
24.8

Scheme 24

pendant double bond, using OsO_4-$NaIO_4$ or $LiIO_4$-OsO_4-buffer, the starting material was destroyed and we failed to obtain the desired **24.8**.

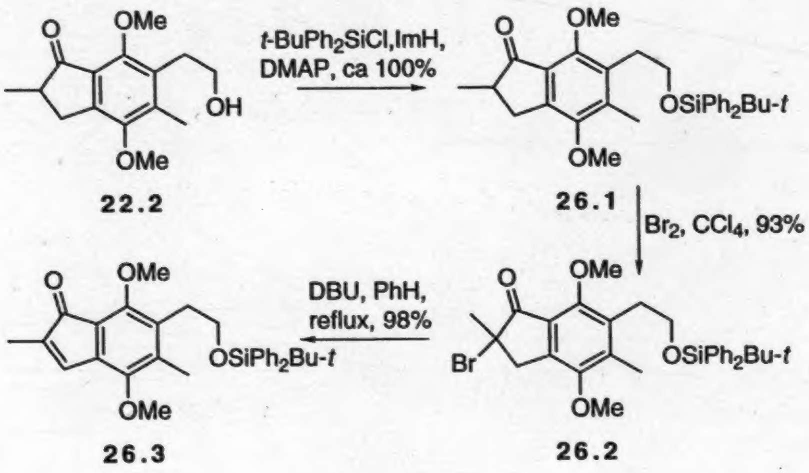
In order to avoid the problem of cleaving the double bond, ketone **16.4** was now dibrominated, so as to afford **24.2** under conditions that gave the compound in 68% yield. Dehydrobromination as before, using DBU, then gave the enone **25.1**. The purpose of having in place the aromatic Br is to provide a means for eventually attaching an oxyethyl sidechain. Treatment of this enone (**25.1**) with (*S*)-CBS-$BH_3.SMe_2$ in PhMe generated the saturated ketone **25.3** instead of the allylic alcohol **25.4**. With CH_2Cl_2 as the solvent, and


Fe, Br2, CCl4, 68%
DBU, PhH, heat, 95%
16.4
24.2
25.1
(S)-CBS (cat), PhMe, BH3.SMe2, 10 °C, 35%
25.1
25.2
(S)-CBS, CH2Cl2, BH3.SMe2 (slow addition at -30 °C), warm to 0 °C
25.1
25.3 (34%)
25.4 (13%)
+ 25.1 (30%)
LiHAl(O-menthyl)3 69%
25.1
25.4 (racemic)
OMe
Br
O
HO

Scheme 25

slow addition of the borane, **25.3**, a small amount of **25.4** (of unestablished chirality), and an appreciable amount of starting **25.1** were obtained. Compound **25.1** is not very soluble in CH_2Cl_2 or PhMe, and this fact limits the conditions that can be used. Although $LiAlH(O\text{-menthyl})_3$ did reduce ketone **25.1** to the allylic alcohol, the material was found to be racemic by ^1H NMR measurements, using a chiral shift reagent. An attempt to make the Mosher ester of **25.4** was unsuccessful.

At this point we decided to work with the oxyethyl sidechain already in place before again attempting to effect an asymmetric reduction of the enone system. We suspected that the solubility problems associated with use of **25.1** could thereby be avoided. We had previously (see Scheme 23) found that an $OSiMe_2Bu$-*t* group was not robust enough to withstand our bromination conditions, and so we took **22.2** and subjected it to silylation with *t*-$BuPh_2SiCl$, and then to bromination, and dehydrobromination, so as to form the enone **26.3**. While we had planned to carry out a thorough investigation on the asymmetric reduction of the ketone carbonyl of **26.3**, another researcher in this laboratory embarked on a more obviously promising approach to the required allylic alcohol. Since his method was successful, we did not continue our work with **26.3**; instead we began the project described in the next chapter.


O
OMe
OH
OMe
t-BuPh2SiCl,ImH,
DMAP, ca 100%
O
OMe
OSiPh2Bu-t
OMe
Br2, CCl4, 93%
O
OMe
Br
OSiPh2Bu-t
OMe
DBU, PhH,
reflux, 98%
O
OMe
OSiPh2Bu-t
OMe

22.2 **26.1** **26.3** **26.2**

Scheme 26

Conclusion

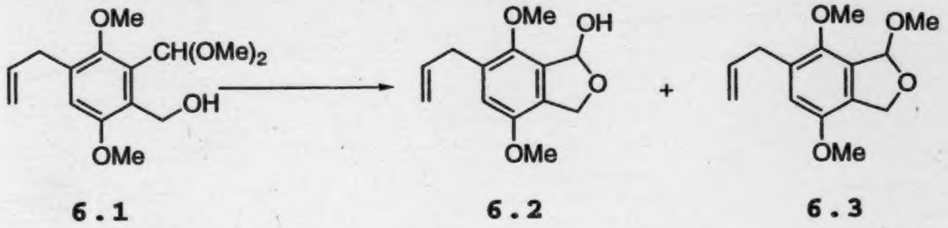
Our research constitutes the first synthesis of puraquinonic acid, for which we have developed two routes. During this work the superiority of $LiIO_4$-OsO_4 in a pH 6.6 buffer over the standard Lemieux-Johnson procedure was demonstrated, as well as the beneficial effect of 2,6-pyridinedicarboxylic acid *N*-oxide on the behavior of $(NH_4)_2Ce(NO_3)_6$ for oxidation of *p*-dimethoxy benzenes.

Our preliminary studies on making optically pure material have revealed some shortcomings of certain standard approaches. In particular the SAMP/RAMP method does not appear to be applicable to sterically hindered ketones. Other work in this laboratory has led to the synthesis of optically pure material and the establishment of the absolute configuration.[30]

EXPERIMENTAL SECTION

General Procedures. Unless stated to the contrary, the procedures described in the Experimental Section of Chapter 1 of this thesis were followed.

1,3-Dihydro-4,7-dimethoxy-6-(2-propenyl)isobenzofuran-1-ol (6.2) and 1,3-Dihydro-1,4,7-trimethoxy-6-(2-propenyl)isobenzofuran (6.3).



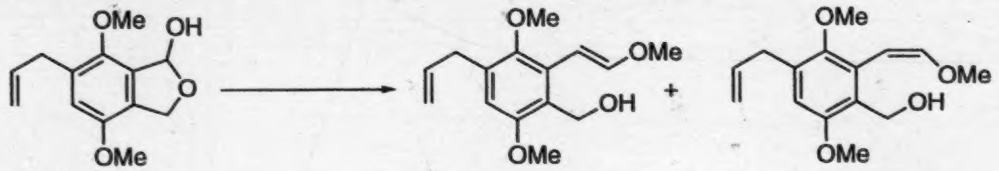
Dilute hydrochloric acid (0.1 M, 10 mL) was added dropwise to a stirred solution of acetal **6.1** (1.304 g, 4.624 mmol) in dioxane (10 mL). Stirring was continued for 12 h, by which time all the starting material had been consumed (TLC control, silica, 2:3 EtOAc-hexane). The mixture was neutralized with saturated aqueous $NaHCO_3$ and the aqueous layer was extracted with CH_2Cl_2, washed with water and brine, dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2.5 x 18 cm), using 1:4 EtOAc-hexane, gave lactol **6.2** (778.1 mg, 71%) as a white solid and the corresponding methyl ether [1,3-dihydro-1,4,7-trimethoxy-6-(2-propenyl)isobenzofuran] (276.7 mg, 24%) as a colorless

oil. Lactol **6.2** had: mp 157.5 °C; FTIR (CH_2Cl_2 cast) 3335 cm^{-1}; ^1H NMR (CD_2Cl_2, 200 MHz) δ 3.31-3.45 (m, 3 H), 3.78 (s, 3 H), 3.82 (s, 3 H), 4.88 (d, *J* = 12 Hz, 2 H), 5.01-5.18 (m, 2 H), 5.88-6.09 (m, 1 H), 6.59 (dd, *J* = 6, 1.8 Hz, 1 H), 6.7 (s, 1 H); ^{13}C NMR (CD_2Cl_2, 100.6 MHz) δ 34.4 (t′), 56.0 (q′), 61.4 (q′), 70.6 (t′), 101.0 (d′), 113.7 (d′), 115.7 (t′), 127.7 (s′), 132.3 (s′), 133.5 (s′), 137.6 (d′), 147.4 (s′), 149.7 (s′); exact mass (HR electrospray) *m/z* calcd for $C_{13}H_{16}NaO_4$ (M + Na) 259.09463, found 259.09467.

1,3-Dihydro-1,4,7-trimethoxy-6-(2-propenyl)isobenzofuran (**6.3**) had: ^1H NMR (CD_2Cl_2, 200 MHz) δ 3.41 (s, 3 H), 3.42 (m, 2 H), 3.76 (s, 3 H), 3.81 (s, 3 H), 4.82-5.15 (m, 4 H), 5.85-6.12 (m, 1 H), 6.25 (d, *J* = 3 Hz, 1 H), 6.68 (s, 1 H); ^{13}C NMR (CD_2Cl_2, 50.3 MHz) δ 35.9 (t′), 55.8 (q′), 57.5 (q′), 62.7 (q′), 72.3 (t′), 108.5 (d′), 115.2 (d′), 117.3 (t′), 129.8 (s′), 132.1 (s′), 134.7 (s′), 139.3 (d′), 149.0 (s′), 151.1 (s′); exact mass (HR electrospray) *m/z* calcd for $C_{14}H_{18}NaO_4$ (M + Na) 273.11028, found 273.10998.

The 1,3-dihydro-1,4,7-trimethoxy-6-(2-propenyl)isobenzofuran (**6.3**) was hydrolyzed to lactol **6.2**, as follows. Dilute hydrochloric acid (0.1 M, 12 mL) was added dropwise to a stirred solution of the lactol methyl ether (1.146 g, 4.58 mmol) in dioxane (10 mL). Stirring was continued for 12 h, by which time all the starting material had been consumed (TLC control, silica, 2:3 EtOAc-hexane). The mixture was neutralized with saturated aqueous $NaHCO_3$ and the aqueous layer was extracted with CH_2Cl_2, washed with water and brine, dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2.5 x 18 cm), using 1:4 EtOAc-hexane, gave lactol **6.2** (737 mg, 68%).

(*E*)- and (*Z*)-[3,6-Dimethoxy-2-(2-methoxyethenyl)-4-(2-propenyl)phenyl]methanol (*E*-6.4) and (*Z*-6.4).[6]



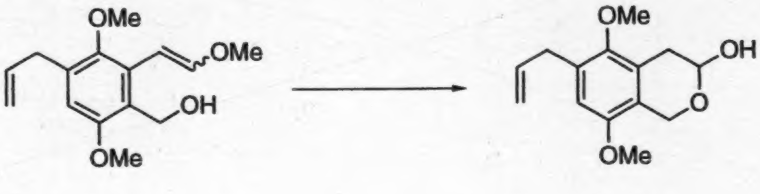
6.2 **E-6.4** **Z-6.4**

(Methoxymethyl)triphenylphosphonium bromide (511.3 mg, 1.483 mmol) was placed in a long-necked flask and dry THF (2 mL) was added. The white slurry was stirred and cooled to -78 °C, and $(Me_3Si)_2NK$ (0.5 M solution in PhMe, 1.7 mL, 0.85 mmol) was added dropwise over 5 min. The resulting red slurry was stirred at -78 °C for 2 h, and a solution of lactol **6.2** (100.0 mg, 0.424 mmol) in dry THF (1 mL plus 1 mL as a rinse) was added dropwise over ca 5 min. The resulting pale orange solution was stirred for 10 h without recharging the cold bath. The resulting white slurry was filtered off using a sintered disc, and washed with EtOAc. Evaporation of the filtrate and flash chromatography of the residue over silica gel (2.5 x 15 cm), using 1:4 EtOAc-hexane, gave the isomeric enol ethers (*E*)-**6.4** (70.8 mg, 63%) and (*Z*)-**6.4** (33.5 mg, 30%) as colorless oils. Compound (*E*)-**6.4** had:

FTIR (CH_2Cl_2 cast) 3462 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 2.16 (t, *J* = 6.9 Hz, 1 H), 3.40 (dt, *J* = 1.4, 6.6 Hz, 2 H), 3.62 (s, 3 H), 3.71 (s, 3 H), 3.84 (s, 3 H), 4.65 (d, *J* = 6.9 Hz, 2 H), 5.05-5.14 (m, 2 H), 5.85 (d, *J* = 15 Hz, 1 H), 5.92-6.05 (m, 1 H), 6.61 (s, 1 H), 6.99 (d, *J* = 15 Hz, 1 H); ^{13}C NMR (CD_2Cl_2, 75.5 MHz) δ 34.7 (t′), 56.1 (q′), 56.7 (q′), 58.0 (t′), 60.4 (q′), 98.0 (d′), 110.1 (d′), 115.9 (t′), 126.0 (s′), 130.8 (s′), 133.4 (s′), 137.7 (d′), 150.1 (s′), 153.1 (d′), 154.9 (s′); exact mass (HR electrospray) *m/z* calcd for $C_{15}H_{20}NaO_4$ (M + Na) 287.12593, found 287.12595.

Compound (*Z*)-**6.4** had: FTIR (CH_2Cl_2 cast) 3462 cm^{-1}; ^1H NMR (CD_2Cl_2, 300 MHz) δ 2.78 (t, *J* = 6.9 Hz, 1 H), 3.40 (dt, *J* = 1.4, 6.6 Hz, 2 H), 3.63 (s, 3 H), 3.64 (s, 3 H), 3.82 (s, 3 H), 4.54 (d, *J* = 6.9 Hz, 2 H), 5.05-5.14 (m, 2 H), 5.41 (d, *J* = 6.8 Hz, 1 H), 5.93-6.23 (m, 1 H), 6.25 (d, *J* = 6.8 Hz, 1 H), 6.67 (s, 1 H); ^{13}C NMR (CD_2Cl_2, 100.6 MHz) δ 34.5 (t′), 56.1 (q′), 59.2 (t′), 60.3 (q′), 61.0 (q′), 100.8 (d′), 111.3 (d′), 116.0 (t′), 127.7 (s′), 129.1 (s′), 133.0 (s′), 137.7 (d′), 148.4 (d′), 150.2 (s′), 154.8 (s′); exact mass (HR electrospray) *m/z* calcd for $C_{15}H_{20}NaO_4$ (M + Na) 287.12593, found 287.12572.

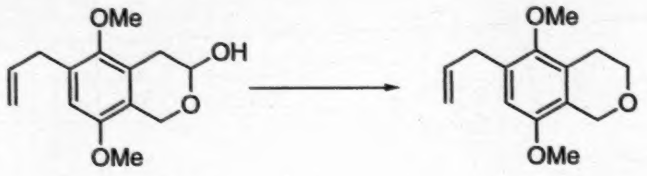
5,8-Dimethoxy-6-(2-propenyl)isochroman-3-ol (6.5).[6]



6.4 **6.5**

Dilute hydrochloric acid (0.1 M, 21.9 mL), was added dropwise to a stirred solution of enol ethers **6.4** (1.822 g, 6.90 mmol) in dioxane (70 mL), and the mixture was then heated at 60 °C for 3 h, by which point all the starting material had reacted (TLC control, silica, 2:3 EtOAc-hexane). The mixture was cooled to room temperature and neutralized with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2, washed with water and brine, dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2.5 x 20 cm), using 1:2 EtOAc-hexane, gave lactol **6.5** (1.500 g, 86%) as a colorless oil: FTIR (CH_2Cl_2 cast) 3404 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 2.71 (dd, *J* = 11.5, 5.2 Hz, 1 H), 3.00 (dd, *J* = 16.6, 3.6 Hz, 1 H), 3.08 (d, *J* = 4.5 Hz, 1 H), 3.39 (d, *J* = 6.6 Hz, 2 H), 3.65 (s, 3 H), 3.76 (s, 3 H), 4.66 (d, *J* = 16 Hz, 1 H), 4.85 (d, *J* = 16 Hz, 1 H), 5.05-5.14 (m, 2 H), 5.22-5.29 (m, 1 H), 5.93-6.03 (m, 1 H), 6.54 (s, 1 H); ^{13}C NMR (CD_2Cl_2, 100.6 MHz) δ 30.0 (t'), 34.4 (t'), 55.7 (q'), 60.7 (t'), 61.0 (q'), 92.4 (d'), 109.4 (d'), 115.8 (t'), 121.7 (s'), 126.2 (s'), 131.5 (s'), 137.8 (d′), 150.0 (s′), 151.6 (s′); exact mass *m/z* calcd for $C_{14}H_{18}O_4$ 250.12051, found 250.11985.

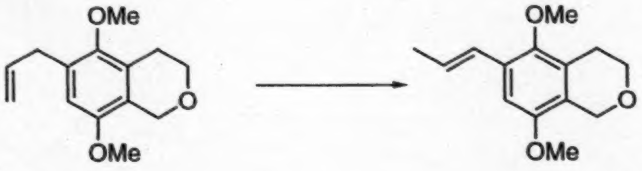
5,8-Dimethoxy-6-(2-propenyl)isochroman (13.1).



6.5 **13.1**

Freshly distilled $BF_3.OEt_2$ (270 μL, 2.12 mmol) was added dropwise to a stirred and cooled (-78 °C) mixture of Et_3SiH[19] (freshly distilled, 462 μL, 2.90 mmol) and lactol **6.5** (482 mg, 1.93 mmol) in dry CH_2Cl_2 (15 mL). After 2 h the cold bath was removed, stirring was continued for 18 h, and the mixture was quenched with saturated aqueous $NaHCO_3$ solution (10 mL). The resulting mixture was extracted with Et_2O, and the combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel, using 2:3 EtOAc-hexanes, gave isochroman **13.1** (423 mg, 93%) as a colorless oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 2.82 (t, *J* = 5.6 Hz, 2 H), 3.42 (d, *J* = 6.5 Hz, 2 H), 3.70 (s, 3 H), 3.77 (s, 3 H), 3.91 (t, *J* = 5.6 Hz, 2 H), 4.70 (s, 2 H), 5.08-5.13 (m, 2 H), 5.94-6.04 (m, 1 H), 6.51 (s, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 23.5 (t′), 34.1 (t′), 55.3 (q′), 60.7 (q′), 64.3 (t′), 64.5 (t′), 108.7 (d′), 115.8 (t′), 122.7 (s′), 128.1 (s′), 130.4 (s′), 137.3 (d′), 149.4 (s′), 151.6 (s′); exact mass *m/z* calcd for $C_{14}H_{18}O_3$ 234.1256, found 234.1249.

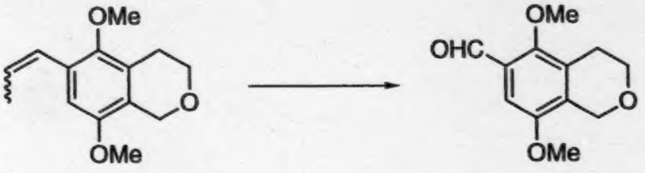
(*E*)-5,8-Dimethoxy-6-(1-propenyl)isochroman (13.2).



13.1 **13.2**

$RhCl_3.3H_2O$ (22.9 mg, 5 mol%) was added to a stirred solution of olefin **13.1** (406 mg, 1.73 mmol) in dry 5:1 PhMe-MeOH (28.8 mL). The mixture was refluxed for 16 h, cooled, and evaporated. Flash chromatography of the residue over silica, using 1:3 EtOAc-hexanes, gave olefin **13.2** (393 mg, 97%) as a white crystalline solid: mp 60-65 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.93 (dd, J = 6.6, 1.6 Hz, 3 H), 2.80 (t, J = 5.6 Hz, 2 H), 3.69 (s, 3 H), 3.80 (s, 3 H), 3.91 (t, J = 5.6 Hz, 2 H), 4.70 (s, 2 H), 6.18-6.27 (m, 1 H), 6.66 (dd, J = 15.9, 1.7 Hz, 1 H), 6.75 (s, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 18.8 (q′), 23.3 (t′), 55.3 (q′), 60.9 (q′), 64.3 (t′), 64.6 (t′), 104.2 (d′), 123.6 (s′), 125.6 (d′), 126.3 (d′), 128.2 (s′), 128.7 (s′), 148.6 (s′), 151.8 (s′); exact mass *m/z* calcd for $C_{14}H_{18}O_3$ 234.1256, found 234.1254.

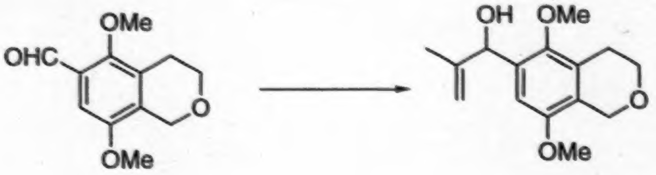
5,8-Dimethoxyisochroman-6-carbaldehyde (13.3).



13.2 **13.3**

OsO_4 (7.0 mg, 5 mol%) was added to a stirred solution of olefins **13.2** (129 mg, 0.551 mmol) in 5:2:2 CCl_4-water-*t*-BuOH (13.5 mL) (the starting material was dissolved in CCl_4-*t*-BuOH, and the water was added last). The mixture was stirred and, after 15 min, $NaIO_4$ (300 mg, 1.38 mmol) was added in one portion. After a further 1.5 h the suspension was diluted with water (5 mL) and extracted with Et_2O. The organic extracts were washed with 10% aqueous $NaHSO_3$ and water, dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (1.8 x 20 cm), using 1:4 EtOAc-hexanes, gave aldehyde **13.3** (117 mg, 97%) as a brown solid: mp 144-149 °C; FTIR ($CDCl_3$ cast) 1677 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.85 (t, J = 5.6 Hz, 2 H), 3.83 (s, 3 H), 3.87 (s, 3 H), 3.93 (t, J = 5.6 Hz, 2 H), 4.74 (s, 2 H), 7.12 (s, 1 H), 10.34 (s, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 22.9 (t′), 55.6 (q′), 63.6 (q′), 64.1 (t′), 64.5 (t′), 104.5 (d′), 126.9 (s′), 129.4 (s′), 132.9 (s′), 152.1 (s′), 155.9 (s′), 189.6 (d′); exact mass *m/z* calcd for $C_{12}H_{14}O_4$ 222.0892, found 222.0893.

(5,8-Dimethoxyisochroman-6-yl)-2-methyl-2-propen-1-ol (13.4).

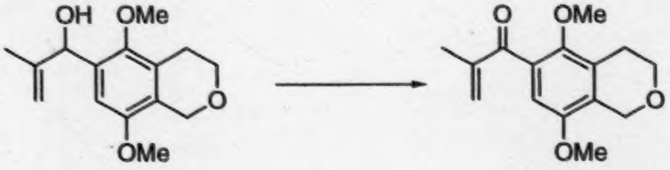


13.3 **13.4**

Isopropenylmagnesium bromide (0.5 M in hexanes, 6.22 mL, 3.89 mmol) was added dropwise to a stirred and cooled (0 °C) solution of aldehyde **13.3** (576 mg, 2.59 mmol) in dry Et_2O (50 mL). After 30 min, the cold bath was removed and stirring was continued for 1 h. The mixture was recooled (0 °C), quenched with saturated aqueous NH_4Cl (10 mL), and taken up in Et_2O (50 mL). The aqueous layer was extracted with Et_2O, and the combined organic extracts were washed with water and brine, dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 1:4 EtOAc-hexanes, gave alcohol **13.4** (590 mg, 86%) as a colorless oil: FTIR ($CDCl_3$ cast) 3417 cm^{-1} (br); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.69 (s, 3 H), 2.40 (br s, 1 H), 2.81 (t, J = 5.5 Hz, 2 H), 3.75 (s, 3 H), 3.79 (s, 3 H), 3.84-3.96 (m, 2 H), 4.64-4.72 (m, 2 H), 5.01-5.03 (m, 1 H), 5.20-5.22 (m, 1 H), 5.41 (s, 1 H), 6.68 (s, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 19.5 (q′), 23.5 (d′), 55.4 (q′), 61.2 (q′), 64.3 (t′), 64.4 (t′), 72.3 (d′), 105.9 (d′), 110.9 (t′), 124.5 (s′), 128.3 (s′), 132.4 (s′), 146.8 (s′), 149.4 (s′), 151.8 (s′); exact mass m/z

calcd for $C_{15}H_{20}O_4$ 264.1362, found 264.1360.

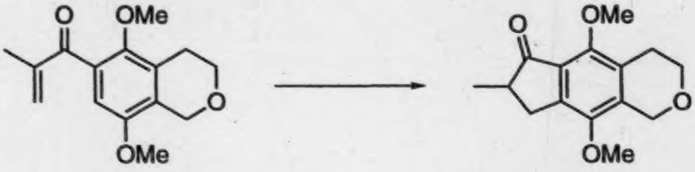
(5,8-Dimethoxyisochroman-6-yl)-2-methyl-2-propen-1-one (13.5).



13.4 **13.5**

Dess-Martin periodinane (80 mg, 0.19 mmol) was added to a stirred solution of allylic alcohol **13.4** (32.6 mg, 0.126 mmol) in dry CH_2Cl_2 (2.5 mL). After 1 h, the mixture was diluted with EtOAc (5 mL) and then stirred for 5 min with saturated aqueous $NaHCO_3$ (2.5 mL) containing $Na_2S_2O_3$ (250 mg). More water (10 mL) was added, and the aqueous layer was extracted with EtOAc. The combined extracts were evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 1:4 EtOAc-hexanes, gave enone **13.5** (32.0 mg, 96%) as a white crystalline solid: mp 90-92 °C; FTIR ($CDCl_3$ cast) 1662 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.06 (s, 3 H), 2.80 (t, J = 5.5 Hz, 2 H), 3.65 (s, 3 H), 3.77 (s, 3 H), 3.92 (t, J = 5.6 Hz, 2 H), 4.71 (s, 2 H), 5.68-5.70 (m, 1 H), 5.95-5.97 (m, 1 H), 6.57 (s, 1 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 17.3 (q'), 23.3 (t'), 55.5 (q'), 62.0 (q'), 64.2 (t'), 64.3 (t'), 107.1 (d'), 127.1 (s'), 128.7 (s'), 129.4 (s'), 130.6 (s'), 144.9 (s'), 149.0 (s'), 151.0 (s'), 198.4 (s'); exact mass *m/z* calcd for $C_{15}H_{18}O_4$ 262.1205, found 262.1203.

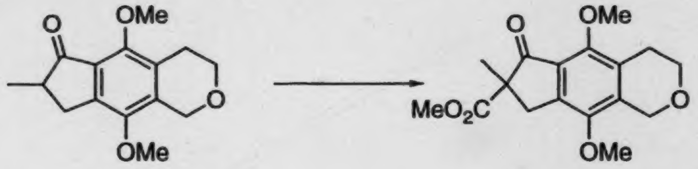
2,3,7,8-Tetrahydro-5*H*-4,9-dimethoxy-2-methyl-6-oxacyclopenta[b]naphthalen-1-one (9.4).



13.5 **9.4**

Cold (0 °C) concentrated H_2SO_4 (0.1 mL) was added to stirred enone **13.5**. The resulting brown solution was stirred for 5 h at 0 °C, diluted with ice-cold water (10 mL) and extracted with Et_2O. The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (1 x 20 cm), using 1:3 EtOAc-hexanes, gave indanone **9.4** (26.6 mg, 83%) as a white crystalline solid: mp 123 °C; FTIR (CH_2Cl_2 cast) 1706 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.25 (d, *J* = 7.2 Hz, 3 H), 2.60-2.71 (m, 2 H), 2.81 (t, *J* = 5.7 Hz, 2 H), 3.38 (q, *J* = 8.8 Hz, 1 H), 3.80 (s, 3 H), 3.86 (t, *J* = 5.8 Hz, 2 H), 3.87 (s, 3 H), 4.81 (s, 2 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 16.5 (q′), 22.9 (t′), 31.7 (t′), 42.4 (d′), 59.8 (q′), 61.4 (q′), 64.6 (d′), 64.7 (d′), 126.8 (s′), 127.4 (s′), 136.3 (s′), 142.2 (s′), 148.4 (s′), 152.0 (s′), 206.0 (s′); exact mass (HR electrospray) *m/z* calcd for $C_{15}H_{18}NaO_4$ (M + Na) 285.11028, found 285.10978.

Methyl 2,3,7,8-Tetrahydro-5*H*-4,9-dimethoxy-2-methyl-1-oxo-6-oxacyclopenta[b]naphthalene-2-carboxylate (13.6).

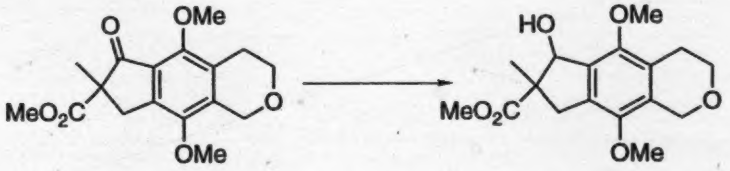


9.4 **13.6**

BuLi (2.5 M in hexanes, 0.74 mL, 1.85 mmol) was added dropwise to a stirred and cooled (-78 °C) solution of *i*-Pr_2NH (278 µL, 1.98 mmol) in THF (5 mL). Stirring was continued for 30 min, and the resulting LDA solution was added dropwise by cannula over ca 10 min to a stirred and cooled (-78 °C) solution of indanone **9.4** (371 mg, 1.42 mmol) in THF (15 mL). Stirring was continued for 40 min and the resulting lithium enolate was quenched with neat MeOC(O)CN (184 µL, 1.98 mmol). After 20 minutes the mixture was transferred to a cold bath at 0 °C, and stirring was continued for 10 min. The mixture was recooled to -78 °C, and saturated aqueous NH_4Cl (5 mL) was added. The aqueous layer was extracted with EtOAc, and the combined organic extracts were washed with water, dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (3 x 15 cm), using 1:4 EtOAc-hexanes, gave ester **13.6** [269 mg, 59% or 82% after correction for recovered starting material (103 mg)] as a colorless liquid: FTIR ($CDCl_3$ cast) 1745, 1709 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.59

(s, 3 H), 2.82 (t, J = 5.7 Hz, 2 H), 2.95 (d, J = 17 Hz, 1 H), 3.66 (d, J = 17 Hz, 1 H), 3.69 (s, 3 H), 3.83 (s, 3 H), 3.92 (t, J = 5.8 Hz, 2 H), 3.94 (s, 3 H), 4.81 (s, 2 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 21.3 (q'), 22.9 (t'), 36.7 (t'), 52.8 (q'), 56.4 (s'), 60.0 (q'), 61.7 (q'), 64.60 (t'), 64.63 (t'), 125.1 (s'), 128.0 (s'), 137.3 (s'), 141.1 (s'), 148.3 (s'), 152.8 (s'), 172.5 (s'), 200.0 (s'); exact mass *m/z* calcd for $C_{17}H_{20}O_6$ 320.1260, found 320.1253.

Methyl 2,3,7,8-Tetrahydro-1-hydroxy-5*H*-4,9-dimethoxy-2-methyl-6-oxacyclopenta[b]naphthalene-2-carboxylate (14.1).

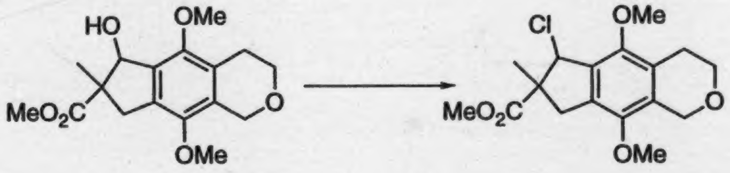


13.6 **14.1**

$NaBH_4$ (95 mg, 2.52 mmol) was added in several portions over 40 min to a stirred and cooled (0 °C) solution of ketone **13.6** in dry MeOH (20 mL). After 1 h at 0 °C, water (2 mL) was added, and the resulting cooled solution was stirred for 30 min, and then extracted with EtOAc. The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 1:24:75 MeOH-EtOAc-hexanes, gave alcohols **14.1** (268 mg, 99%) as a colorless oil: FTIR ($CHCl_3$ cast) 3430, 1729 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) (mixture of diastereoisomers) δ 1.25 (s, 0.71), 1.35 (s, 2.4 H), 1.59 (s, 0.91 H), 2.60 (d, *J* = 7.8 Hz, 0.69 H), 2.68-2.86 (m, 2 H), 2.92 (d, *J* = 15.8 Hz, 0.8 H), 3.37 (d, *J* = 15.8 Hz, 0.8 H), 3.73-3.96 (m including three s, 10.5 H in all), 4.73 (s, 2 H), 5.09 (d, *J* = 4.5 Hz, 0.16 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) (mixture of diastereoisomers) δ 18.3 (q′), 23.2 (t′), 23.3 (t′), 23.4 (q′), 37.2 (t′), 38.7 (t′), 52.3 (q′), 52.4 (q′), 55.2 (t′), 55.4 (t′), 59.8 (q′), 60.6 (q′), 61.4 (q′), 64.6 (t′), 64.7 (t′), 77.8 (d′), 79.3 (d′), 126.7 (s′), 126.9 (s′), 129.0 (s′), 129.5 (s′), 129.7 (s′), 131.2 (s′), 132.3 (s′), 133.0 (s′), 148.4 (s′), 150.7 (s′), 151.3 (s′), 176.9 (s′); exact mass *m/z* calcd for $C_{17}H_{22}O_6$ 322.1416, found 322.1413.

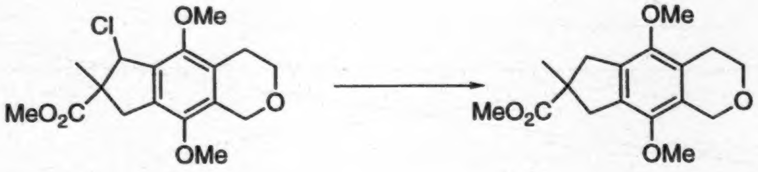
Methyl 1-Chloro-2,3,7,8-tetrahydro-5*H*-4,9-dimethoxy-2-methyl-6-oxacyclopenta[b]naphthalene-2-carboxylate (14.2).



14.1 **14.2**

$SOCl_2$ (121 μL, 1.66 mmol) was added dropwise to a stirred and cooled (0 °C) solution of alcohols **14.1** (268 mg, 0.832 mmol) and Et_3N (232 μL, 1.66 mmol) in dry CH_2Cl_2 (5 mL). After 30 min the cold bath was removed, and the mixture was refluxed for 4 h. The mixture was cooled and poured into water (10 mL). The aqueous layer was extracted with EtOAc, and the combined organic extracts were washed with water, dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (1 x 20 cm), using 1:5 EtOAc-hexanes, gave chlorides **14.2** [153 mg, 54% or 93% after correction for recovered starting material (114 mg)] as a colorless liquid: FTIR ($CHCl_3$ cast) 1737 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) (mixture of diastereoisomers) δ 1.31 (s, 1.4 H), 1.61 (s, 2.0 H), 2.69-2.90 [m including d (J = 20.6 Hz) at δ 2.85, 2.46 H in all], 2.96 (d, J = 15.6 Hz, 0.59 H), 3.60-3.65 [m including s and d (J = 20.6 Hz), 2.3 H in all], 3.75-4.00 [m including singlets at δ 3.78, 3.81, 3.83, 3.89, 3.90 and d at δ 3.82 (J = 15.6 Hz), 9.4 H in all], 4.68-4.79 (m, 2 H), 5.27 (s, 0.38 H), 5.86 (s, 0.46 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) (mixture of diastereoisomers) δ 21.2 (q'), 23.3 (t'), 23.8 (q'), 36.7 (t'), 37.9 (t'), 52.3 (q'), 52.8 (q'), 56.9 (t'), 57.4 (t'), 59.8 (q'), 59.9 (q'), 60.8 (q'), 60.9 (q'), 64.58 (t'), 64.61 (t'), 65.9 (d'), 66.0 (d'), 127.1 (s'), 127.2 (s'), 130.1 (s'), 130.2 (s'), 130.4 (s'), 131.1 (s'), 132.2 (s'), 133.8 (s'), 147.9 (s'), 149.0 (s'), 149.8 (s'), 150.4 (s'), 173.7 (s'), 175.4 (s'); exact mass m/z calcd for $C_{17}H_{21}{}^{35}ClO_5$ 340.1078, found 340.1080.

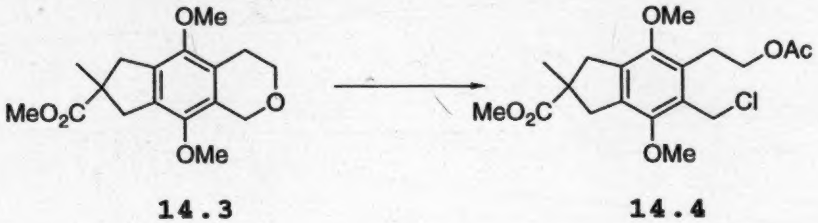
Methyl 2,3,7,8-Tetrahydro-5*H*-4,9-dimethoxy-2-methyl-6-oxacyclopenta[b]naphthalene-2-carboxylate (14.3).



14.2 **14.3**

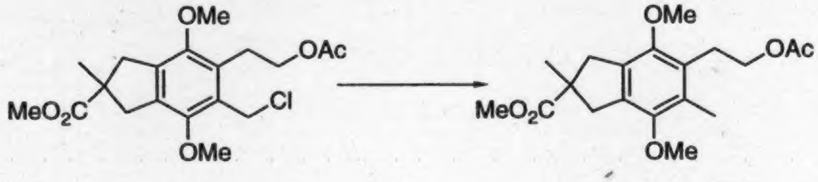
A mixture of chloride **14.2** (138 mg, 0.405 mmol), Bu_3SnH (220 μL, 0.810 mmol) and AIBN (10 mg, 0.061 mmol) in dry PhMe (5 mL) was refluxed for 3 h. The mixture was cooled and applied to a column of silica gel (1 x 20 cm), which was developed successively with hexanes, 1:20 EtOAc-hexanes, 1:10 EtOAc-hexanes and 1:5 EtOAc-hexanes, to give **14.3** as a colorless oil contaminated with tin residues. Flash chromatography over silica gel, using 1:5 EtOAc-hexanes, gave ester **14.3** (118 mg, 95%) as a colorless oil: FTIR ($CHCl_3$ cast) 1731 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.36 (3 H), 2.76 (t, J = 5.6 Hz, 2 H), 2.87 (d, J = 15.7 Hz, 2 H), 3.46 (dd, J = 15.8, 3.0 Hz, 2 H), 3.73-3.74 (overlapping singlets at δ 3.73, 3.735, 3.74, 9 H in all), 3.83-3.94 (m, 2 H), 4.74 (s, 2 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 23.2 (t'), 25.0 (q'), 41.0 (t'), 41.2 (t'), 50.1 (t'), 52.2 (q'), 59.6 (q'), 59.7 (q'), 64.5 (t'), 64.7 (t'), 125.8 (s'), 126.9 (s'), 130.7 (s'), 131.9 (s'), 148.3 (s'), 150.0 (s'), 177.7 (s'); exact mass m/z calcd for $C_{17}H_{22}O_5$ 306.1467, found 306.1464.

Methyl 6-[2-(Acetyloxy)ethyl]-5-chloromethyl-4,7-dimethoxy-2-methylindan-2-carboxylate (14.4).



$ZnCl_2$ (5.5 mg, 10 mol%) and AcCl (82 μL, 1.16 mmol) were added to a stirred solution of isochroman **14.3** (118 mg, 0.39 mmol) in dry CH_2Cl_2 (6 mL), and the mixture was refluxed (Ar atmosphere) for 6 h. The solvent was then evaporated, and flash chromatography of the residue over silica gel (1.8 x 20 cm), using 1:4 EtOAc-hexanes, gave acetate **14.4** (131 mg, 88%) as a colorless oil: FTIR ($CDCl_3$ cast) 1736 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.36 (s, 3 H), 2.05 (s, 3 H), 2.89 (dd, J = 16.1, 2.8 Hz, 2 H), 3.05 (t, J = 7.2 Hz, 2 H), 3.47 (d, J = 10.3 Hz, 1 H), 3.51 (d, J = 10.5 Hz, 1 H), 3.74 (s, 3 H), 3.78 (s, 3 H), 3.84 (s, 3 H), 4.21 (t, J = 7.6 Hz, 2 H), 4.76 (AB q, $\Delta\nu_{AB}$ = 25.5 Hz, J = 10.9 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 21.0 (q′), 25.0 (q′), 26.1 (t′), 38.2 (t′), 41.2 (t′), 41.6 (t′), 50.1 (t′), 52.2 (q′), 60.2 (q′), 60.9 (q′), 64.1 (t′), 128.9 (s′), 133.5 (s′), 135.2 (s′), 151.2 (s′), 151.6 (s′), 170.9 (s′), 177.5 (s′) (two signals in this spectrum overlap); exact mass m/z calcd for $C_{19}H_{25}{}^{35}ClO_6$ 384.1340, found 384.1337.

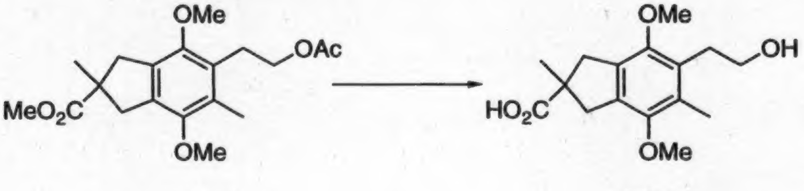
Methyl 6-[2-(Acetyloxy)ethyl]-4,7-dimethoxy-2,5-dimethylindan-2-carboxylate (14.5).



14.4 **14.5**

Bu_3SnH (136 μL, 0.51 mmol) and AIBN (7 mg, 15 mol%), were added to a stirred solution of chloride **14.4** (97.8 mg, 0.25 mmol) in PhMe (6 mL), and the mixture was refluxed for 1.5 h. Evaporation of the solvent and flash chromatography of the residue over silica gel (1.8 x 12 cm), using 1:9 EtOAc-hexanes, gave acetate **14.5** contaminated with tin residues. Flash chromatography over silica gel (1.8 x 12 cm), using 1:9 EtOAc-hexanes, gave acetate **14.5** (77.3 mg, 87%) as a colorless oil: FTIR ($CDCl_3$ cast) 1735 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.35 (s, 3 H), 2.05 (s, 3 H), 2.23 (s, 3 H), 2.87 (dd, J = 15.4, 4.0 Hz, 2 H), 2.96 (dt, J = 7.7, 2.2 Hz, 2 H), 3.46 (dd, J = 15.8, 12.9 Hz, 2 H), 3.69 (s, 3 H), 3.73 (s, 3 H), 3.76 (s, 3 H), 4.14 (t, J = 7.7 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 12.1 (q′), 21.1 (q′), 25.2 (q′), 26.6 (t′), 41.1 (t′), 41.6 (t′), 50.1 (t′), 52.2 (q′), 60.0 (q′), 60.2 (q′), 63.8 (t′), 127.9 (s′), 129.3 (s′), 131.1 (s′), 133.3 (s′), 150.9 (s′), 151.5 (s′), 171.1 (s′), 177.8 (s′); exact mass m/z calcd for $C_{19}H_{26}O_6$ 350.1730, found 350.1727.

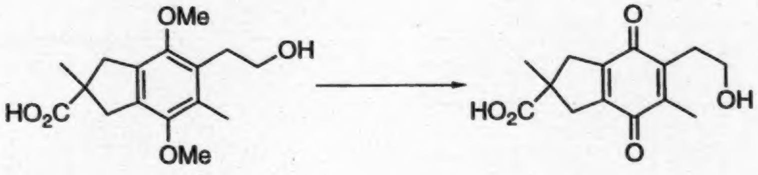
Methyl 6-(2-Hydroxyethyl)-4,7-dimethoxy-2,5-dimethylindan-2-carboxylate (14.6).



14.5 **14.6**

$LiOH.H_2O$ (28.0 mg, 0.66 mmol) was added to a stirred solution of ester **14.5** (15.3 mg, 0.044 mmol) in 1:1 dioxane-water (4 mL). After 3 h, the mixture was acidified with hydrochloric acid (1.0 M, 4 mL) and then extracted with EtOAc. The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (0.8 x 20 cm), using 1:19 $MeOH-CH_2Cl_2$, gave alcohol **14.6** (11.6 mg, 90%) as a white solid: FTIR ($CHCl_3$ cast) 1701 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.41 (s, 3 H), 2.09 (s, 1 H), 2.21 (s, 3 H), 2.86-2.94 (m, 4 H), 3.47-3.55 (m, 2 H), 3.70 (s, 3 H), 3.73-3.80 (m containing s at δ 3.76, 5 H in all), 3.87 (d, J = 11.7 Hz, 1 H), 3.92 (d, J = 11.6 Hz, 1 H), 4.11 (s, 3 H), 3.72-3.80 (m including s at δ 4.17, 5 H in all); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 12.2 (q′), 25.0 (q′), 30.5 (t′), 41.1 (t′), 41.5 (t′), 50.0 (t′), 60.0 (q′), 60.2 (q′), 62.7 (t′), 129.1 (s′), 129.2 (s′), 131.2 (s′), 133.0 (s′), 151.1 (s′), 151.2 (s′), 183.2 (s′); exact mass m/z calcd for $C_{16}H_{22}O_5$ 294.1467, found 294.1467.

2,3,4,7-Tetrahydro-5-(2-hydroxyethyl)-2,6-dimethyl-4,7-dioxo-1*H*-indene-2-carboxylic acid (puraquinonic acid) (1.1).



14.6 **1.1**

An ice-cold solution of $Ce(NH_4)_2(NO_3)_6$ (277 mg, 0.506 mmol) in 1:1 MeCN-water (0.8 mL) was added slowly to a stirred and cooled (0 °C) solution of alcohol **14.6** (45.8 mg, 0.156 mmol) in 2:1 MeCN-water (0.9 mL) containing pyridine-2,6-dicarboxylic acid *N*-oxide (92.7 mg, 0.506 mmol). After 40 min, the mixture was diluted with water (5 mL), and extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography over silica gel (1.8 x 20 cm), using CH_2Cl_2, gave quinone **1.1** (31.7 mg, 77%) as a brown liquid with ^1H and ^{13}C NMR identical, within experimental error, with the reported[1] values.

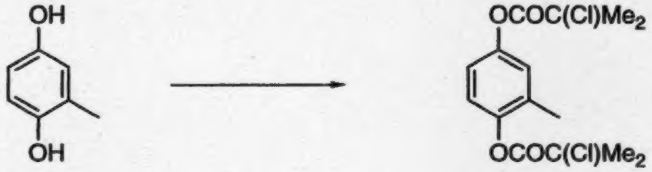
Table 1 (^1H NMR spectrum of puraquinonic acid)

Natural	Natural	Synthetic	Synthetic
$CDCl_3$, 500 MHz		$CDCl_3$, 300 MHz	
1.41	s, 3 H	1.41	s, 3 H
2.07	s, 3 H	2.06	s, 3 H
2.74	m, 2 H	2.69-2.80	m, 4 H
2.78	t, *J* = 6.5 Hz, 2 H	-	-
3.37	m, 2 H	3.32-3.41	m, 2 H
3.75	t, *J* = 6.5 Hz, 2 H	3.74	t, *J* = 6.3 Hz, 2 H

Table 2 (^{13}C NMR spectrum of puraquinonic acid)

Natural	**Synthetic**
$CDCl_3$, 125 MHz	$CDCl_3$, 50 MHz
12.1	12.1
25.7	25.6
29.9	29.8
42.3	42.2
42.3	-
46.9	46.9
61.4	61.3
141.4	141.3
142.8	142.8
145.4	145.4
145.7	145.7
181.5	181.8
185.7	185.7
186.2	186.2

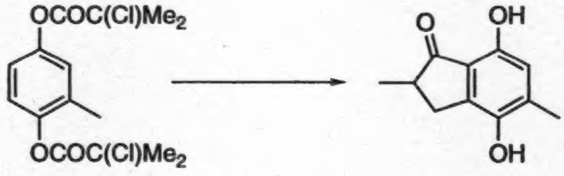
1,4-Phenylene 2-Chloro-2-methylpropanoate (16.2).



16.1 **16.2**

α-Chloroisobutyroyl chloride (8.774 g, 62.2 mmol) was added dropwise 20 min, to a stirred and cooled (0 °C) solution of 2-methylhydroquinone **16.1** (3.513 g, 28.3 mmol) in pyridine (24 mL). The cold bath was removed and stirring was continued for 12 h. The pyridine was evaporated and the residue was dissolved in Et_2O (300 mL), washed with hydrochloric acid (1.0 M, 3 x 100 mL) and water (100 mL), and dried ($MgSO_4$). Evaporation of the solvent gave crude diester **16.2** (8.372 g, 88%) as a brown solid which was used for the next step without purification: mp 52-58 °C; FTIR ($CDCl_3$ cast) 1758 cm^{-1}; ^{13}C NMR ($CDCl_3$, 50.3 MHz) (mixture of rotamers) δ 16.2 (q′), 16.3 (q′), 18.9 (q′), 19.0 (q′), 29.6 (q′), 29.7 (q′), 34.2 (q′), 64.3 (s′), 119.3 (d′), 119.9 (d′), 122.2 (d′), 122.4 (d′), 122.6 (d′), 122.8 (d′), 123.4 (d′), 123.6 (d′), 123.8 (d′), 124.0 (d′), 131.4 (s′), 131.8 (s′), 146.4 (s′), 146.8 (s′), 147.1 (s′), 148.0 (s′), 148.3 (s′), 169.7 (s′), 170.0 (s′), 175.1 (s′), 175.5 (s′); exact mass *m/z* calcd for $C_{15}H_{18}{}^{35}Cl_2O_4$ 332.0582, found 332.0586.

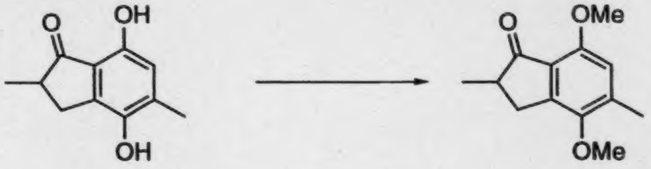
4,7-Dihydroxy-2,5-dimethylindan-1-one (16.3).



16.2 **16.3**

Diester **16.2** (3.626 g, 10.9 mmol) and anhydrous $AlCl_3$ (5.782 g, 43.4 mmol) were thoroughly mixed and heated at 125 °C for 20 min and at 190 °C for 10 min. The mixture was cooled to room temperature, and the resulting solid was coarsely powdered and poured onto ice (100 g) containing concentrated hydrochloric acid (20 mL). The mixture was extracted with Et_2O (5 x 100 mL) and the combined organic extracts were back-extracted with aqueous 10% NaOH (3 x 100 mL). The alkaline solution was acidified with concentrated hydrochloric acid and extracted with Et_2O (3 x 100 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2.5 x 15 cm), using 1:4 EtOAc-hexanes, gave indanone **16.3** (900 mg, 43%), which was recrystallized from hot $CHCl_3$ as yellow crystals: mp 134-136 °C (lit.[23] 124-125 °C); FTIR ($CHCl_3$ cast) 1657, 3386 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.31 (d, J = 7.4 Hz, 3 H), 2.29 (s, 3 H), 2.58 (dd, J = 17.0, 3.5 Hz, 1 H), 2.74-2.86 (m, 1 H), 3.27 (dd, J = 17.1, 7.7 Hz, 1 H), 4.76 (s, 1 H), 6.59 (s, 1 H), 8.47 (s, 1 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 16.3 (q′), 16.8 (q′), 31.5 (t′), 42.2 (d′), 115.97 (d′), 116.0 (d′), 120.4 (s′), 137.2 (s′), 143.8 (s′), 151.0 (s′), 211.6 (s′); exact *m/z* calcd for $C_{11}H_{12}O_3$ 192.0786, found 192.0787.

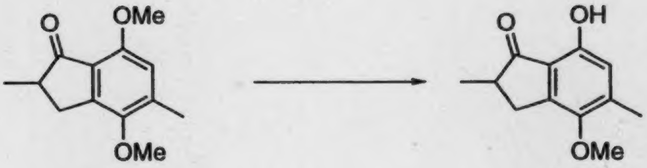
4,7-Dimethoxy-2,5-dimethylindan-1-one (16.4).



16.3 **16.4**

MeI (4.03 mL, 64.7 mmol) was added dropwise to a stirred mixture of phenol **16.3** (1.243 g, 6.47 mmol) and K_2CO_3 (8.943 g, 64.7 mmol) in dry DMF (20 mL). The mixture was warmed to 70 °C, stirred for 5 h at this temperature, poured into brine, and extracted with Et_2O (4 x 75 mL). The combined organic extracts were washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2.5 x 20 cm), using 1:5 EtOAc-hexanes, gave methyl ether **16.4** (914 mg, 64%) as a colorless oil: FTIR ($CDCl_3$ cast) 1707 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.27 (d, J = 7.3 Hz, 3 H), 2.34 (s, 3 H), 2.59-2.67 (m, containing a doublet of a doublet at 2.62, J = 18.6, 3.8 Hz, 2 H in all), 3.28-3.35 (m, 1 H), 3.76 (s, 3 H), 3.88 (s, 3 H), 6.59 (s, 1 H); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 16.7 (q'), 16.8 (q'), 31.5 (t'), 42.3 (q'), 55.8 (q'), 60.0 (d'), 111.9 (d'), 123.5 (s'), 139.9 (s'), 147.2 (s'), 148.7 (s'), 153.9 (s'), 206.3 (s'); exact mass m/z calcd for $C_{13}H_{16}O_3$ 220.1099, found 220.1101.

7-Hydroxy-4-methoxy-2,5-dimethylindan-1-one (19.1).

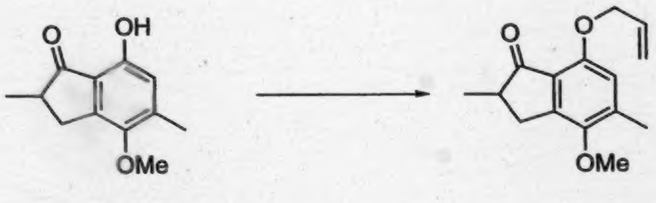


16.4 **19.1**

BCl_3 (1.0 M in hexanes, 12.0 mL, 12.0 mmol) was added dropwise to a stirred and cooled (-78 °C) solution of methyl ether **16.4** (879 mg, 3.99 mmol) in dry CH_2Cl_2 (40.0 mL). The resulting pale yellow solution was stirred for 5 h without recharging the cold bath. The solution was recooled to 0 °C, water (15 mL) was added slowly, and the resulting mixture was extracted with EtOAc (4 x 50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 1:5 EtOAc-hexanes, gave phenol **19.1** (802 mg, 97%) as a brown liquid: FTIR ($CDCl_3$ cast) 3342, 1675 cm^1; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.30 (d, *J* = 7.4 Hz, 3 H), 2.29 (s, 3 H), 2.69 (dd, *J* = 17.3, 3.6 Hz, 1 H), 2.71-2.79 (m, 1 H), 3.38 (dd, *J* = 17.3, 7.6 Hz, 1 H), 3.77 (s, 3 H), 6.58 (s, 1 H), 8.73 (s, 1 H); irradiation of the CH_3 signal at δ 3.77 resulted in a 1% enhancement of the CH_3 signal at δ 2.29 and a 2% enhancement of the CH_2 signal at δ 2.69); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 16.2 (q′), 16.9 (q′), 32.4 (t′), 41.9 (t′), 60.0 (d′), 116.0 (d′), 120.6 (s′), 141.8 (s′), 143.0 (s′), 148.1

(s′), 153.0 (s′) (carbonyl signal not observed); exact mass *m/z* calcd for $C_{12}H_{14}O_3$ 206.0943, found 206.0945.

2,5-Dimethyl-4-methoxy-7-(2-propenyloxy)indan-1-one (19.2).

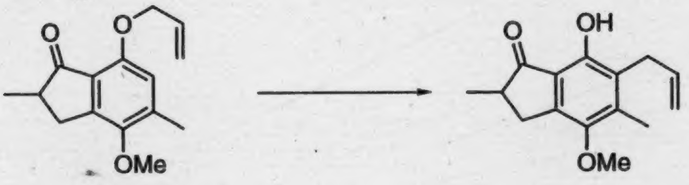


19.1 **19.2**

A solution of phenol **19.1** (802 mg, 3.89 mmol) in dry DMF (14.0 mL) was added dropwise to a stirred and cooled (0 °C) suspension of NaH (95%, 108 mg, 4.28 mmol) in dry DMF (10.0 mL). The cold bath was removed and stirring was continued for 1 h. The mixture was recooled (0 °C) and allyl bromide (neat, 674 μL, 7.79 mmol) was added dropwise over 10 min. The cold bath was removed and stirring was continued for 1.5 h, and the mixture was poured into brine (50 mL) and extracted with Et_2O (3 x 50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 1:6 EtOAc-hexanes, gave allyl ether **19.2** (916 mg, 96% or 99% for recovered **19.1**, 26 mg) as a colorless liquid: FTIR ($CDCl_3$ cast) 1706, 1602 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.21 (d, J = 7.3 Hz, 3 H), 2.26 (s, 3 H), 2.27-2.61 (m, 2 H), 3.22-3.30 (m, 1 H), 3.70 (d, J = 0.7 Hz, 3 H), 4.56 (dd, J =

4.9, 0.8 Hz, 2 H), 5.22 (dt, *J* = 10.6, 1.2 Hz, 1 H), 5.46 (dt, *J* = 17.3, 1.2 Hz, 1 H), 5.94-6.05 (m, 1 H), 6.53 (s, 1 H); ^{13}C NMR (CDCl$_3$, 50.3 MHz) δ 16.7 (two q′), 31.5 (t′), 42.4 (q′), 60.0 (q′), 69.4 (t′), 113.6 (d′), 117.6 (t′), 124.0 (s′), 132.7 (d′), 139.6 (s′), 147.0 (s′), 148.9 (s′), 152.9 (s′), 206.0 (s′); exact mass *m/z* calcd for $C_{15}H_{18}O_3$ 246.1256, found 246.1264.

7-Hydroxy-2,5-dimethyl-4-methoxy-6-(2-propenyl)-indan-1-one (19.3).

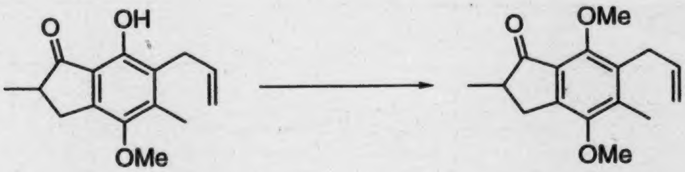


19.2 **19.3**

A solution of allyl ether **19.2** (793 mg, 3.03 mmol) in degassed decalin (5.0 mL) was refluxed for 8 h (N_2 atmosphere), and then cooled to room temperature. The mixture was loaded onto a dry silica gel column (2 x 15 cm), and flash chromatography, using hexanes and then 1:5 EtOAc-hexanes, gave **19.3** (536 mg, 67% or 75% after correction for recovered **19.2**, 85 mg) as a colorless oil: FTIR (CDCl$_3$ cast) 2933, 1674 cm^{-1}; ^{1}H NMR (CDCl$_3$, 360 MHz) δ 1.31 (d, *J* = 7.4 Hz, 3 H), 2.27 (s, 3 H), 2.68 (dd, *J* = 17.2, 3.6 Hz, 1 H), 2.75-2.80 (m, 1 H), 3.38 (dd, *J* = 17.2, 7.6 Hz, 1 H), 3.42 (m, 2 H), 3.76 (s, 3 H), 4.93-5.02 (m, 2 H), 5.86-5.96 (m, 1

H), 9.04 (s, 1 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 12.8 (q′), 16.2 (q′), 29.5 (t′), 32.2 (t′), 41.9 (d′), 60.2 (q′), 115.0 (t′), 119.9 (s′), 124.7 (s′), 135.0 (d′), 140.9 (s′), 141.0 (s′), 148.1 (s′), 151.4 (s′), 211.6 (s′); exact mass *m/z* calcd for $C_{15}H_{18}O_3$ 246.1256, found 246.1260.

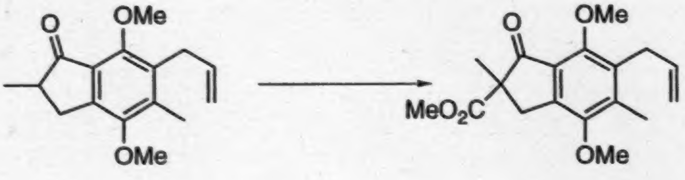
4,7-Dimethoxy-2,5-dimethyl-6-(2-propenyl)indan-1-one (19.4).



19.3 **19.4**

MeI (0.66 mL, 10.5 mmol) was added dropwise to a stirred mixture of phenol **19.3** (514 mg, 2.10 mmol) and K_2CO_3 (1.456 g, 10.5 mmol) in dry DMF (20 mL). The mixture was warmed to 70 °C, stirred for 6 h at this temperature, poured into brine (40 mL), and extracted with Et_2O (4 x 40 mL). The combined organic extracts were washed with water (40 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 1:10 EtOAc-hexanes and then 1:5 EtOAc-hexanes, gave **19.4** (504 mg, 93%) as a colorless oil: FTIR ($CHCl_3$ cast) 1707 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.29 (d, *J* = 7.3 Hz, 3 H), 2.28 (s, 3 H), 2.62-2.72 (m, 2 H), 3.32-3.40 (m, 1 H), 3.44-3.47 (m, 2 H), 3.78 (s, 3 H), 3.91 (s, 3 H), 4.90 (dq, *J* = 17.1, 1.8 Hz, 1 H), 5.00 (dq, J = 10.2, 1.7 Hz, 1 H), 5.85-5.96 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 12.8 (q′), 16.5 (q′), 30.5 (t′), 31.6 (t′), 42.4 (q′), 60.1 (q′ or d′), 62.3 (d′ or q′), 115.1 (t′), 126.8 (s′), 132.0 (s′), 136.0 (d′), 139.2 (s′), 144.6 (s′), 151.2 (s′), 152.5 (s′), 206.0 (s′); exact mass m/z calcd for $C_{16}H_{20}O_3$ 260.1412, found 260.1407.

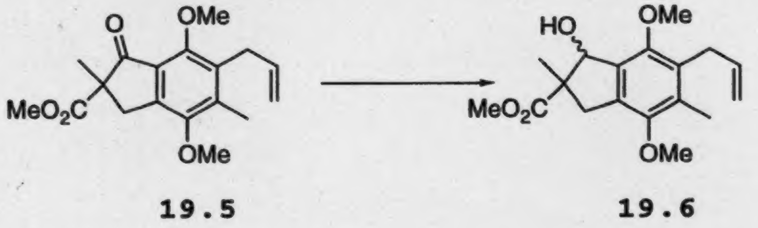
Methyl 4,7-Dimethoxy-2,5-dimethyl-1-oxo-6-(2-propenyl)indan-2-carboxylate (19.5).



19.4 **19.5**

BuLi (2.5 M in hexanes, 300 μL, 0.75 mmol) was added to a stirred and cooled (-78 °C) solution of i-Pr_2NH (115 μL, 0.82 mmol) in dry THF (1.0 mL). Stirring was continued for 30 min, and the resulting LDA solution was added dropwise by syringe over 10 min to a stirred and cooled (-78 °C) solution of indanone **19.4** (175 mg, 0.68 mmol) in THF (2.50 mL). Stirring was continued for 40 min, and the resulting enolate was quenched with neat MeOC(O)CN (82 μL, 1.02 mmol). After 1 h at -78 °C, saturated aqueous NH_4Cl (10 mL) was added, and the mixture was extracted with EtOAc (3 x 25 mL). The combined organic extracts were washed with water (30 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (1.5 x 18 cm), using 1:5 EtOAc-hexanes, gave **19.5** (173 mg, 79% or 87% corrected for recovered **19.4**, 14.0 mg) as a colorless oil: FTIR ($CDCl_3$ cast) 1745, 1707 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.52 (s, 3 H), 2.30 (s, 3 H), 2.94 (d, *J* = 17.3 Hz, 1 H), 3.46-3.48 (m, 2 H), 3.64 (d, *J* = 17.3 Hz, 1 H), 3.70 (s, 3 H), 3.79 (s, 3 H), 3.91 (s, 3 H), 4.89-4.92 (m, 1 H), 5.01-5.05 (m, 1 H), 5.86-5.96 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 12.8 (q'), 21.2 (q'), 30.6 (t'), 36.6 (t'), 52.6 (q'), 56.4 (s'), 60.2 (q'), 62.3 (q'), 115.3 (t'), 125.1 (s'), 132.6 (s'), 135.8 (d'), 140.2 (s'), 143.6 (s'), 151.1 (s'), 153.2 (s'), 172.6 (s'), 200.0 (s'); exact mass *m/z* calcd for $C_{18}H_{22}O_5$ 318.1467, found 318.1466.

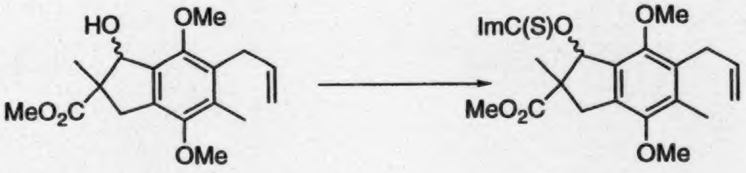
Methyl 1-Hydroxy-4,7-dimethoxy-2,5-dimethyl-6-(2-propenyl)indan-2-carboxylate (19.6).



$NaBH_4$ (65 mg, 1.64 mmol) was added in small portions over 30 min to a stirred and cooled (0 °C) solution of ketone **19.5** (173 mg, 0.546 mmol) in dry MeOH (6.0 mL). After 40 min, water (1 mL) was added, stirring was continued for 0.5 h at 0 °C, and the resulting mixture was extracted with EtOAc

(4 x 20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (1.5 x 15 cm), using 1:5 EtOAc-hexanes, and then 1:14:85 MeOH-EtOAc-hexanes, gave alcohols **19.6** (159 mg, 91%) as a colorless oil: FTIR ($CDCl_3$, cast) 3459, 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) (mixture of diastereoisomers) δ 1.26 (s, 0.68 H), 1.35 (s, 2.29 H), 1.60 (br s, 0.24 H), 2.17 (s, 2.28 H), 2.19 (s, 0.68 H), 2.64 (br s, 0.64 H), 2.80 (d, J = 16.0 Hz, 0.42 H), 2.91 (d, J = 15.9 Hz, 0.78 H), 3.31-3.53 [m containing d at δ 3.35 (J = 15.8 Hz), 2.81 H in all], 3.64-3.74 [overlapping signals: d at δ 3.66 (J = 16.1 Hz) and singlets at δ 3.70, δ 3.73 and δ 3.74, 5.62 H in all), 3.80-3.81 (two overlapping singlets, 3 H in all], 3.88 (s, 0.67 H), 4.89-4.92 (m, 1 H), 4.99-5.02 (m, 1 H), 5.08 (s, 0.21 H), 5.63 (s, 0.75 H), 5.87-5.98 (m, 0.95 H); ^{13}C NMR ($CDCl_3$, 125.7 MHz) (mixture of diastereoisomers) δ 12.1 (q′), 18.4 (q′), 23.5 (q′), 30.9 (t′), 31.1 (t′), 37.1 (t′), 38.7 (t′), 52.2 (q′), 52.3 (q′), 55.3 (s′), 60.0 (q′), 61.4 (q′), 62.6 (q′), 78.2 (d′), 79.8 (d′), 114.9 (d′), 115.0 (d′), 131.0 (s′), 131.1 (s′), 131.2 (s′), 131.4 (s′), 132.0 (s′), 132.1 (s′), 132.4 (s′), 133.0 (s′), 136.25 (d′), 136.30 (d′), 150.86 (s′), 150.90 (s′), 150.95 (s′), 151.2 (s′), 151.3 (s′), 152.0 (s′), 176.0 (s′), 176.8 (s′); exact mass m/z calcd for $C_{18}H_{24}O_5$ 320.1624, found 320.1627.

Methyl 1-(1*H*-Imidazol-1-ylthiomethoxy)-4,7-dimethoxy-2,5-dimethyl-6-(2-propenyl)indan-2-carboxylate (19.7).

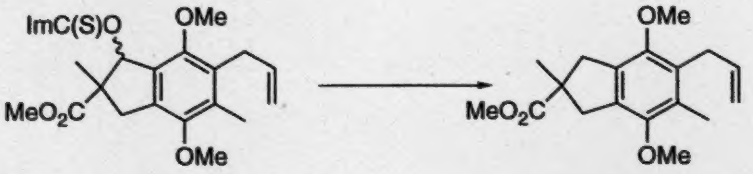


19.6 **19.7**

1,1′-Thiocarbonyldiimidazole (214 mg, 1.20 mmol) was added to a stirred mixture of alcohol **19.6** (127 mg, 0.40 mmol) and DMAP (5 mg, 10 mol%) in dry 1,2-dichloroethane (2.0 mL). Stirring was continued overnight by which time all **19.6** had reacted. The mixture was diluted with CH_2Cl_2 (15 mL), washed with water (3 x 10 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (1.5 x 20 cm), using 1:3 EtOAc-hexanes, gave imidazolide **19.7** (167 mg, 97%) as a white crystalline solid: mp 127-136 °C; FTIR ($CDCl_3$ cast) 1732, 1694 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) (mixture of diastereoisomers) δ 1.51 (s, 0.76 H), 1.56 (s, 2.32 H), 2.17 (s, 2.29 H), 2.19 (s, 0.76 H), 2.84-2.92 [overlapping doublets at δ 2.87 (J = 16.2 Hz) and δ 2.89 (J = 16.5 Hz), 1 H in all], 3.19-3.54 (m, 2 H), 3.60-3.65 (m, 1.73 H), 3.71-3.79 [overlapping signals: singlets at δ 3.71, δ 3.72 and δ 3.79 and d at δ 3.76 (J = 17.3 Hz), 8.5 H in all], 4.88-4.93 (m, 1 H), 5.00-5.02 (m, 1 H), 5.37 (s, 0.20 H), 5.84-5.95 (m, 1 H), 5.99 (s, 0.70 H), 7.08-7.09 [overlapping doublets at δ 7.08

(J = 1.4 Hz) and δ 7.09 (J = 1.0 Hz), 0.92 H in all], 7.44 (s, 0.21 H), 7.48 (s, 0.68 H), 8.18 (s, 0.20 H), 8.21 (s, 0.70 H); ^{13}C NMR ($CDCl_3$, 125.7 MHz) (mixture of diastereoisomers) δ 12.2 (q′), 20.7 (q′), 25.0 (q′), 31.1 (t′), 38.4 (t′), 39.6 (t′), 52.0 (q′), 52.8 (q′), 54.9 (q′), 55.5 (q′), 55.6 (s′), 56.3 (s′), 60.10 (q′), 60.13 (d′), 61.7 (q′), 61.9 (d′), 115.2 (t′), 115.9 (d′), 129.0 (s′), 129.6 (s′), 130.8 (d′), 131.9 (s′), 132.0 (s′), 132.2 (s′), 132.6 (s′), 132.8 (s′), 133.0 (s′), 135.9 (d′), 150.6 (s′), 150.9 (s′), 151.4 (s′), 164.6 (s′), 165.3 (s′)k, 173.9 (s′), 175.7 (s′); exact mass (HR electrospray) m/z calcd for $C_{22}H_{26}N_2NaO_5S$ 453.1460, found 453.1460.

Methyl 4,7-Dimethoxy-2,5-dimethyl-6-(2-propenyl)-indan-2-carboxylate (19.8).

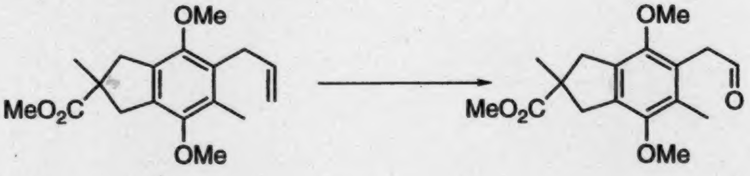


19.7 **19.8**

A mixture of imidazolide **19.7** (173 mg, 0.402 mmol), Bu_3SnH (220 μL, 0.804 mmol) and AIBN (10 mg, 15 mol%) in dry PhMe (5.0 mL) was refluxed for 1.5 h. The solvent was evaporated and flash chromatography of the residue over silica gel (1.5 x 18 cm), using 1:5 EtOAc-hexanes, gave **19.8** as a colorless oil contaminated with tin residues. Flash

chromatography over silica gel, using 1:5 EtOAc-hexanes, gave **19.8** (101 mg, 83%) as a pure, colorless oil: FTIR ($CHCl_3$ cast) 1733 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.36 (s, 3 H), 2.16 (s, 3 H), 2.88 (dd, *J* = 16.0, 2.2 Hz, 2 H), 3.38-3.50 [m containing dd at δ 3.46 (*J* = 15.8, 4.7 Hz), 4 H in all], 3.70 (s, 3 H), 3.72 (s, 3 H), 3.73 (s, 3 H), 4.89-4.93 (m, 1 H), 4.98-5.02 (m, 1 H), 5.89-5.97 (m, 1 H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 11.8 (q′), 25.2 (q′), 31.0 (t′), 41.2 (t′), 41.4 (t′), 50.0 (s′), 52.1 (q′), 59.9 (q′), 60.4 (q′), 114.7 (t′), 129.1 (s′), 130.3 (s′), 131.4 (s′), 132.5 (s′), 136.7 (d′), 151.0 (s′), 177.9 (s′), two signals overlap in this spectrum; exact mass *m/z* calcd for $C_{18}H_{24}O_4$ 304.1675, found 304.1679.

Methyl 4,7-Dimethoxy-2,5-dimethyl-6-(2-oxoethyl)-indan-2-carboxylate (19.9).



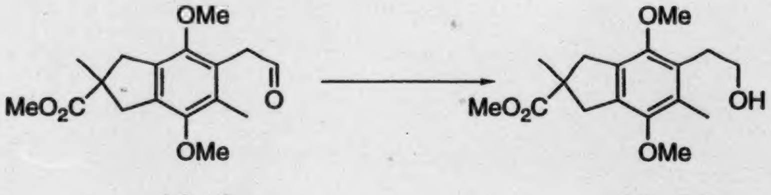
19.8 **19.9**

Li_3PO_4 buffer (pH 7.0) was prepared from 0.2 M H_3PO_4 and sufficient solid $LiOH.H_2O$ to bring the pH to 7.0 (pH meter).

A solution of $LiIO_4$ and Li_3PO_4 at pH 6.6 was then made by dissolving HIO_4 (2.279 g, 10.0 mmol) in the pH 7.0 Li_3PO_4 buffer (50.0 mL), and adjusting the resulting solution to pH 6.6 with $LiOH.H_2O$.

Aqueous OsO_4 (1.0 w/v%, 40 μL, 0.002 mmol) and the $LiIO_4$-Li_3PO_4 solution (0.79 mL) were added to a stirred solution of olefin **19.8** (10.5 mg, 0.035 mmol) in EtOAc (0.79 mL). Stirring was continued for 17 h (N_2 atmosphere). The mixture was diluted with EtOAc (15 mL), washed with water (3 x 5 mL) and brine (5 mL), dried ($MgSO_4$), and evaporated to give crude aldehyde **19.9** (10.4 mg, 98%) as a pale brown liquid (we assume the color is due to traces of osmium species): FTIR ($CDCl_3$ cast) 1727 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.37 (s, 3 H), 2.12 (s, 3 H), 2.89 (d, *J* = 15.9 Hz, 1 H), 2.91 (d, *J* = 15.9 Hz, 1 H), 3.47 (d, *J* = 15.8 Hz, 1 H), 3.50 (d, *J* = 15.8 Hz, 1 H), 3.71 (overlapping singlets, 6 H in all), 3.72 (t, *J* = 2.0 Hz, 2 H), 3.74 (s, 3 H), 9.68 (t, *J* = 2.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 12.6 (q′), 25.1 (q′), 30.9 (q′), 41.2 (t′), 41.6 (t′), 42.5 (t′), 50.1 (s′), 52.2 (q′), 60.0 (q′), 123.5 (s′), 129.5 (s′), 131.1 (s′), 134.3 (s′), 151.0 (s′), 151.2 (s′), 177.7 (s′), 199.6 (d′); exact *m/z* calcd for $C_{17}H_{22}O_5$ 306.1467, found 306.1472.

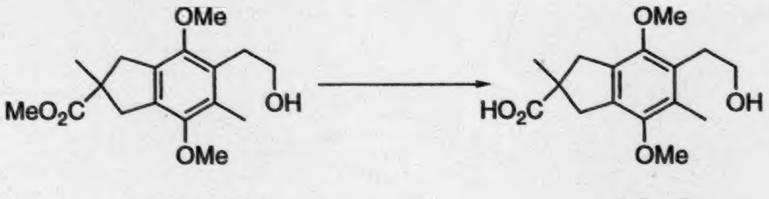
Methyl 5-(2-Hydroxyethyl)-4,7-dimethoxy-2,6-dimethylindan-2-carboxylate (19.10).



19.9 **19.10**

$NaBH_4$ (4.6 mg, 0.12 mmol) was added in three equal portions, to a stirred and cooled (0 °C) solution of aldehyde **19.9** (12.4 mg, 0.041 mmol) in dry MeOH (2.0 mL). When all the starting material had reacted (ca 0.5 h, TLC control, silica, 1:3 EtOAc-hexanes), water (0.5 mL) was added, and stirring was continued for 0.5 h at 0 °C. The solvent was evaporated and the residue was taken up in EtOAc (15 mL), washed with water (3 x 10 mL) and dried ($MgSO_4$). Evaporation of the solvent and flash chromatography of the residue over silica gel, using 1:4 EtOAc-hexanes, gave alcohol **19.10** (12.1 mg, 96%) as a colorless oil: FTIR ($CDCl_3$ cast) 3427, 1731 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.36 (s, 3 H) 1.90 (br s, 1 H), 2.21 (s, 3 H), 2.84-2.97 [m containing a dd at δ 2.87 (J = 16.1, 3.3 Hz) and a t at δ 2.92 (J = 6.8 Hz), 4 H in all), 3.44 (d, J = 15.9 Hz, 1 H), 3.48 (d, J = 15.8 Hz, 1 H), 3.70 (s, 3 H), 3.73-3.79 (m, containing two singlets at δ 3.73 and δ 3.76, 8 H in all); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 12.2 (q′), 25.3 (q′), 30.6 (t′), 41.2 (t′), 41.6 (t′), 50.1 (s′), 52.2 (q′), 60.0 (q′), 60.1 (q′), 62.8 (t′), 128.9 (two overlapping s′), 131.0 (s′), 132.9 (s′), 150.98 (s′), 151.09 (s′), 177.6 (s′); exact mass m/z calcd for $C_{17}H_{24}O_5$ 308.1624, found 308.1619.

5-(2-Hydroxyethyl)-4,7-dimethoxy-2,6-dimethylindan-2-carboxylic Acid (14.6).



19.10 **14.6**

LiOH•H_2O (23.1 mg, 0.55 mmol) was added to a stirred solution of ester **19.10** (11.3 mg, 0.037 mmol) in 1:1 dioxane-water (2 mL). After 3 h, the mixture was washed with CH_2Cl_2 (5.0 mL), acidified with concentrated hydrochloric acid, and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (0.8 x 15 cm), using 1:19 MeOH-CH_2Cl_2, gave **14.6** (10.1 mg, 93%) as a white solid, identical to material made by our previous[22] route.

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CHAPTER 3

SYNTHESIS OF DERIVATIZED AMINO ACIDS FOR PROTEIN SYNTHESIS BY NATIVE CHEMICAL LIGATION

Notation and abbreviations

In the following review, structures of the type HLSSMERVEWLRKKLQDVHNF are normally written as H_2N-HLSSMERVEW-LRKKLQDVHNF-OH, although in the protein literature it is usual to omit the "H_2N-" and "-OH" at the amino and carboxyl termini, respectively.

Formulas of the type H_2N-SKAL$^{\alpha}$COSH, H_2N-SKAL-COSH, or H_2N-SKAL-SH should be taken to mean that the carboxyl terminus (in this case, leucine) has its carboxyl in the form of a thioacid. The usual formulation in the protein literature would be SKAL-SH, and we have used this notation sometimes.

Both three-letter and one-letter symbols for the amino acids are used, the latter especially for long sequences. For selenocysteine the abbreviations are "Sec" and "U".

The symbols $A_1 ... A_n$ refer to amino acid residues numbered 1 to n, and the designation $A_1 ... A_n$-CO_2H indicates a free carboxyl on residue n. Likewise, the designation $P_1P_2 ... P_n$ refers to peptide segments numbered 1 to n.

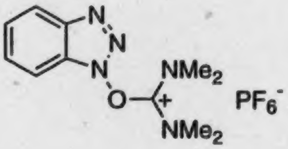
Abbreviations

Acm = acetamidomethyl = $CH_2NHCOMe$ (used as a sulfur protecting group: $SCH_2NHCOMe$)

Dnp = 2,4-dinitrophenyl = protection for histidine

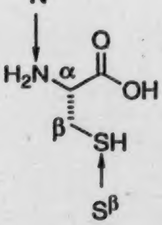
BOP = benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate

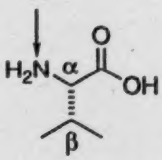
HBTU = 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (Note that the so-called uronium salt is, in fact, a guanidinium *N*-oxide (see Fluka *Peptide and Peptidomimetic Synthesis*; Fluka Chemie Gmbh: Buchs, 2000, page 80).



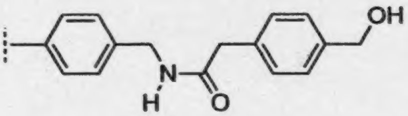
Msc = 2-(methylsulfonyl)ethoxycarbonyl = $MeSO_2CH_2CH_2OCO$-; protection for N^{α}

Amino acids labels are assigned according to the following system:

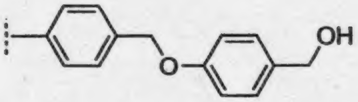




PAM resin:



WANG resin:



Introduction

Attempts to synthesize very large peptides of any sequence and of lengths up to those characteristic of proteins is an important endeavor because the development of practical methods for this purpose would have significant consequences. One can predict that the relationships between sequence and function could be identified so that artificial enzymes could be made to perform specific chemical operations. The availability of such enzymes would certainly change current industrial practice.

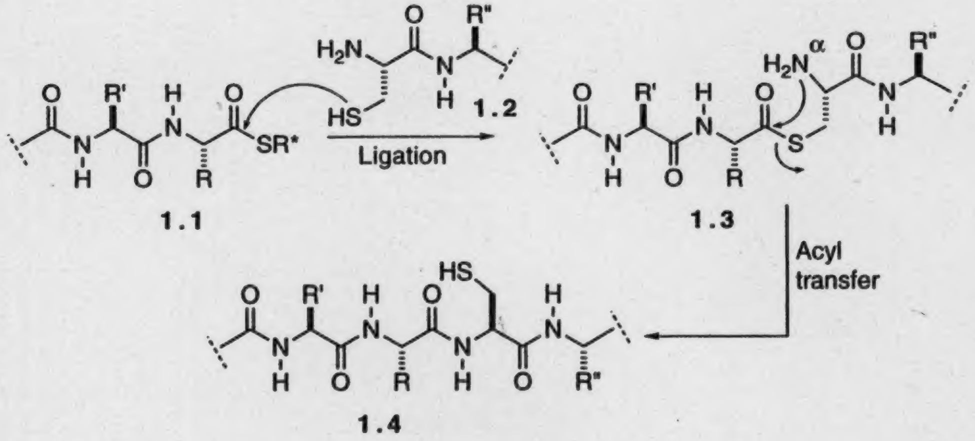
A great deal of work has been done in the area of large peptide synthesis.[1] With solid phase methods, peptides up to about 70 residues (statements in the literature vary from ca 50 to ca 100) in length can be assembled, but larger peptides (especially, over 100 residues[2]) are not available[3,4] because of the increasing proportions of impurities resulting from incomplete acylation at each of the individual steps. Assembly of large peptides by *simple coupling* of two shorter peptides is also not a generally practical approach, since the required activation of the carboxy terminus (for reaction with the amino terminus of the second peptide) causes epimerization at the adjacent asymmetric center, unless[5,6] the carboxyl terminus is Gly or Pro. Recently, coupling conditions proceeding with low *C*-terminal epimerization have been identified for coupling of peptide segments of up to 21 amino acid units.[7] The helpful effect of substituting the *C*-terminal backbone amide nitrogen has also been investigated (see later in the section on *Use of amide nitrogen backbone protection*). Such protection improves yields in coupling single amino acids to build up certain sequences that otherwise give poor yields, and can also be used in coupling of protected segments, because *N*-substitution also suppress epimerization of a *C*-terminal amino acid when it is activated. However, it appears that this approach has not yet been adequately tested with large peptides.

In recent years much progress has been made to solve the problems of assembling very large peptides. These methods rely on an initial reaction that proceeds well under conditions of low concentration and that serves to link two peptides together. This ligation step is then followed by an acyl transfer that forms the peptide bond. The subject has been reviewed at length by D. Coltart[8] while he worked in this laboratory on the problem of peptide ligation, and only the currently most promising methods are described here. A number of methods for joining two large peptides together, followed by formation of a peptide bond, have been developed, but only a few of them lead to a native peptide – the others result in the formation of an unnatural unit at the site of ligation,[8] and are not dealt with in this summary.

Native chemical ligation

The most promising technique for linking large peptides is the process of *native chemical ligation*.[9] This was reported in 1994 (Scheme 1) by Dawson, Muir, Clark-Lewis, and

Kent,[10] and is based on the following principle:[11] An unprotected peptide α-thioester (**1.1**) is treated with an

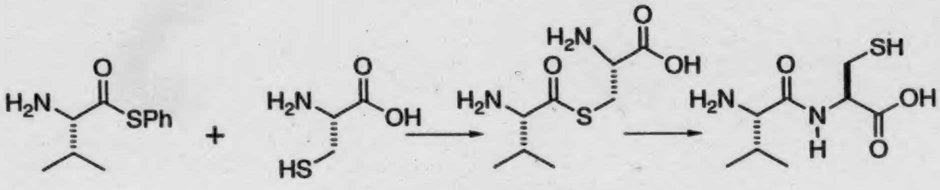


Scheme 1

unprotected peptide whose amino terminus is a Cys residue. Ligation occurs by thioester exchange (**1.1** + **1.2** → **1.3**), and this step, which produces a thioester-linked intermediate (**1.3**), is followed by spontaneous acyl migration (**1.3** → **1.4**). The result is that the two initial peptide segments have been joined to afford a much larger peptide with a native backbone.

A related acyl transfer is actually used in nature for the process of protein splicing, which involves the self-catalyzed excision of an intervening polypeptide – the intein – from an inactive enzyme precursor, and the formation of an active enzyme by joining the flanking regions by a peptide bond.[12]

The process summarized in Scheme 1 has a number of characteristics. Unprotected peptides are used, thus circumventing the complications inherent in the classical use of combinations of protecting groups that lead to limited solubility of many intermediates. The initial ligation is based on the fact that thiols react readily with thioesters, even under conditions of low dilution.[13] The reaction is chemoselective so that unprotected peptides can be used, as the side-chain functional groups do not interfere. In practice, the reaction is usually run in the presence of an excess of the thiol corresponding to the thioester leaving group (or in the presence of PhSH) so as to keep Cys residues in the reduced (i.e. SH) form without interfering with the ligation. Even internal Cys residues may be present;[14] they can undergo ester exchange with the peptide α-thioester component, but this reaction is unproductive because no rearrangement to the peptide bond can occur. Formation of the thus-formed thioester is easily reversed so that reactions at internal Cys have no permanent effect on the outcome. The $S \rightarrow N$ acyl migration occurs spontaneously, and the intermediate **1.3** is usually not observed. The facility of this rearrangement results from the favorable geometric arrangement of the N^{α}-moiety with respect to the thioester unit in **1.3**. Such "entropy activation", as it is called, was first suggested by Brenner.[15] In addition, thiol esters show a special reactivity towards amine nucleophiles.[16] The high intramolecular acylating power of the thioester functionality was first observed by Wieland,[17] who treated **2.1** with Cys (**2.2**) and obtained **2.4** (Scheme 2).



2.1 **2.2** **2.3** **2.4**

Scheme 2

The thioester terminus of the *N*-terminal peptide (**1.1**) is not very highly activated, and so epimerization does not occur. In a test[2] with model peptides, using ligation at a Leu-Cys site, the presence of D-Leu in the ligation product was not detectable and was judged to be less than 1%. Racemization at a His-Cys ligation site was reported to be less than 2% in another publication.[18]

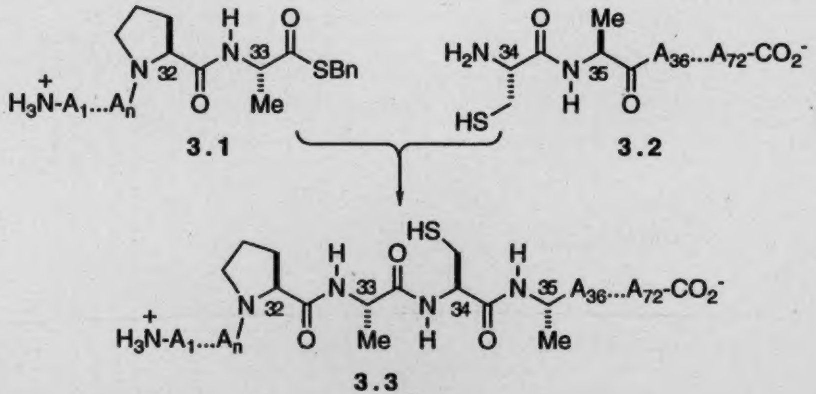
The method of native chemical ligation[10] does requires that ligation be at a Cys residue; this is a significant limitation, as Cys is not common in proteins.[19] Best results are obtained if the thioester terminus is unhindered, i.e. a non-β-branched amino acid is preferred. However, for coupling between a thioester terminus $-X^{\alpha}COSR$ and H_2N-$CysA_1...A_n$, amino acid X can, in fact, have any value except Pro.[20] If X = Thr,[18] Val,[18] Ile[18] or Tyr[22] ligation rates are low. For X = Cys or His, the rate is the same as for X = Gly.[18]

Solubilizing agents such as urea or guanidinium hydrochloride do not interfere with the ligation and rearrangement. Use of improved thioester leaving groups (i.e. variation in the nature of SR* in **1.1**) leads to faster ligation (SPh faster than SBn).

A number of model studies, carried out by Tam and his colleagues,[23] established that very effective conditions for transthioesterification involved the addition not only of a thiol but also of a phosphine, conveniently the water-soluble phosphine $(HO_2CCH_2CH_2)_3P$, which appears to accelerate thioester exchange. In the simple cases studied [e.g. Boc-Gly$^{\alpha}COSCH_2CH_2CO_2H$ + H_2N-Cys-Phe-Lys-Ala-OH] yields of the desired product Boc-Gly-Cys-Phe-Lys-Ala-OH were very high (>90%) after a reaction period of 8 h at pH 7.2. Acylation of the nitrogen was also found to be specific for the N^{α} amino group and the ε-*N* of lysine was not acylated.

Synthesis of proteins and large polypeptides

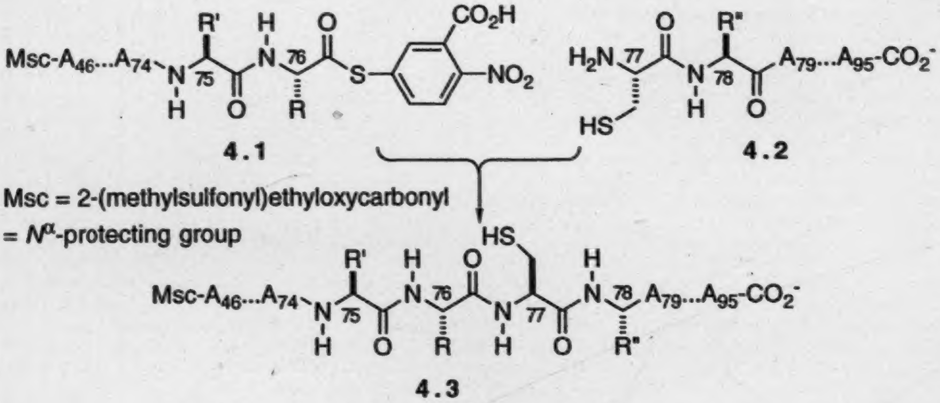
Application of the principle of native chemical ligation to the total synthesis of a protein was initially illustrated by the preparation[10] of [Ala^{33}]-human interleukin 8 – a 72-amino acid polypeptide mutant compound that has full biological activity.



Scheme 3

The two unprotected synthetic peptide segments **3.1** and **3.2**, prepared by solid phase methods, reacted cleanly in a phosphate buffer at pH 7.6 in the presence of guanidine hydrochloride and BnSH to give the full length polypeptide chain of [Ala^{33}]-interleukin 8 in reduced (i.e. SH) form. It is noteworthy that each of the segments contains two Cys residues, but these do not interfere with the ligation-acyl transfer.

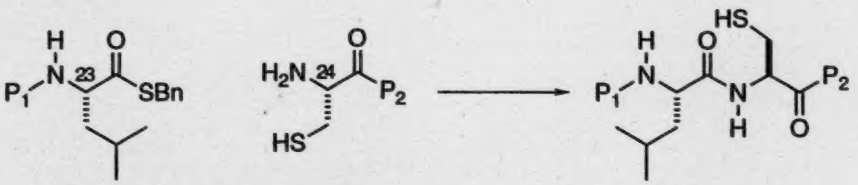
Another peptide – residues 46-95 from the external domain of the human interleukin-3 receptor β-subunit – was also prepared, but in this case (Scheme 4) the thioester was made with a better leaving group (see **4.1**). Reaction occurred at pH 5 in an ammonium acetate buffer.


4.1
4.2
4.3
Msc = 2-(methylsulfonyl)ethyloxycarbonyl
= N^{α}-protecting group

Scheme 4

Turkey ovomucoid third domain (OMTKY3)[24] is a potent protein inhibitor of certain serine proteinases.[2] The segment (6-56)OMTKY3[24] was synthesized by solid phase peptide synthesis, using Boc-chemistry, and by native chemical ligation in order to compare these two different approaches.

The *C*-terminal segment (24-56)OMTKY3 has the sequence (written in the amino → carboxyl direction) C^{24}GSDNKTYGNKCNFCNAVVESNGTLTLSHFGKC^{56}. The *N*-terminal peptide representing residues 6-23 has the sequence (also written in the amino → carboxyl direction) V^{6}DCSEYPKPACTLEYRPL^{23} and was made in the form (6-23)$^{\alpha}$COSBn. Both compounds were prepared by solid phase peptide synthesis, the benzyl ester being generated by alkylation of the corresponding thioacid with BnBr. Ligation under standard conditions [6 M guanidinium hydrochloride, pH 7.5, 1% BnSH, 3% PhSH, 0.025 M in each peptide segment, 36 h] gave (6-56)OMTKY3. The above transformations are summarized in Scheme 5.



5.1 **5.2** **5.3**

$P_1 = H_2N\text{-}V^{6}DCSEYPKPACTLEYRP^{22}$

$P_2 = HN\text{-}G^{25}SDNKTYGNKCNFCNAVVESNGTLTLSHFGKC^{56}\text{-}OH$

Scheme 5

BnSH present in the ligation reaction mixture acted as a reducing agent to prevent formation of disulfide bonds, both inter- and intramolecular. Its presence would also convert nonproductive ligated thioesters (from internal Cys) back into starting materials. In cases such as the present one, where the thioester has a bulky side chain (see **5.1**), *in situ* transthioesterification with PhSH enhanced the ligation rate. The two chemical approaches (solid phase synthesis and native chemical ligation) gave comparable yields in this particular case, but the peptide involved is small – only 56 amino acid residues – and the ligation method is expected to show its superiority in the synthesis of larger targets.

Bovine pancreatic trypsin inhibitor – a 58 amino acid protein – was made in a similar way to OMTKY3, the segments used being H_2N-RPDFCLEPPYTGPCKARIIRYFYNAKAGLCQTFVYGG-$^{\alpha}COSCH_2CO_2H$ and H_2N-CRAKRNNFKSAEDCMRTCGGA-OH.[25]

In connection with studies of a transmembrane protein, the 19-amino acid segment H_2N-KKKSTWVLVGGVLAALAAY$^{\alpha}COSR$ and the 47-amino acid segment H_2N-CLTTGSVVIVGRIILSGRPAVIPDREVLYQEFDEMEECASHLPYKKKK-$CONH_2$ have also been linked[22] under the standard conditions in the presence of PhSH. The reaction was slow – the thioester terminus is derived from a Tyr unit – and was only 50% complete after 90 h, at which point reaction was stopped. The yield of coupled native peptide was 40% after correction for recovered starting peptides, which could be reused.

A modified version of chymotrypsin inhibitor 2, a 64-amino acid protein, was prepared in like manner from (1-40)$^{\alpha}COSR$ and H_2N-Cys(41-64) segments.[21]

Peptide synthesis with the phosphine-thiol method[23,26] was used to make a number of peptides ranging from 9 to 54 amino acid residues (Scheme 6[23]). Again, unprotected internal Cys does not interfere with the ligation-acyl transfer

	Thioester	***N*-Terminal cysteinyl peptide**	**Yield**
1	H_2N-SRDFG-SR*	H_2N-CAKA-OH	88%
2	H_2N-GERGAL-SR*	H_2N-CFKA-OH	87%
3	H_2N-AVSEINFMHNLGKHLSS-SR*	H_2N-CDHARHGFLPRHRDTGILDSC(Acm)A-OH	60%
4	H_2N-PQITLWQRPLVTIRIGGQLKEALLDTGADDTVLEEMN-SR*	H_2N-CHSGYVGARCEHADLLA-OH	60%

SR* = $SCH_2CH_2CO_2H$; Acm = $CH_2NHC(O)Me$ (protecting group for cysteinyl sulfur)

Scheme 6

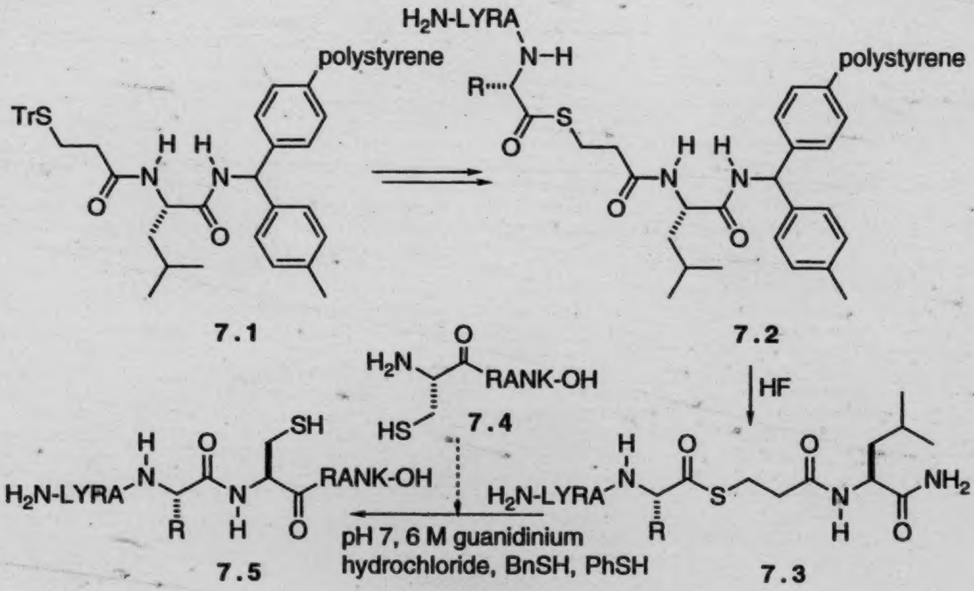
sequence (example #4).

Control of reactivity of thioesters

The reactivity of the thioester can be altered *in situ*[11] since peptide α-thioesters undergo transthioesterification[4] when exposed to thiols. Thus, peptides containing a benzyl α-thioester can be converted into the more reactive phenyl α-thioester by addition of thiophenol to the ligation mixture.[4,11] In a test case,[4] synthetic peptides corresponding to barnase(1-48)$^{\alpha}$COSBn and the analog sequence [Cys^{49},His^{80},Ala^{102}]barnase(49-110) were ligated in the presence of either BnSH or PhSH. After 7 h the reaction involving PhSH was essentially complete, but with BnSH the process was only 25% complete. Peptides corresponding to barnase(1-48)$^{\alpha}$COSBn and the analog sequence [Cys^{49}]barnase(49-110) were also ligated in the presence of PhSH.[4] Formation of [Cys^{49}]barnase was essentially complete after 4.5 h at pH 7.5.

Preparation of thioesters and effect of varying the thioester terminus

In order to facilitate the preparation of thioesters, a resin and linker system (see **7.1** and **7.2**) was designed that is compatible with the standard conditions used for Boc-chemistry solid phase peptide synthesis.[18] After cleavage from the resin, the resulting thioester **7.3** can be used directly for native chemical ligation (**7.3** → **7.5**). A variety of peptide thioesters H_2N-LYRAX$^{\alpha}$COSR, where X$^{\alpha}$COSR represents the thioester of any amino acid, were prepared and ligated with H_2N-CRANK-OH (**7.4**, Scheme 7) to yield H_2N-LYRAXCRANK-OH (**7.5**).

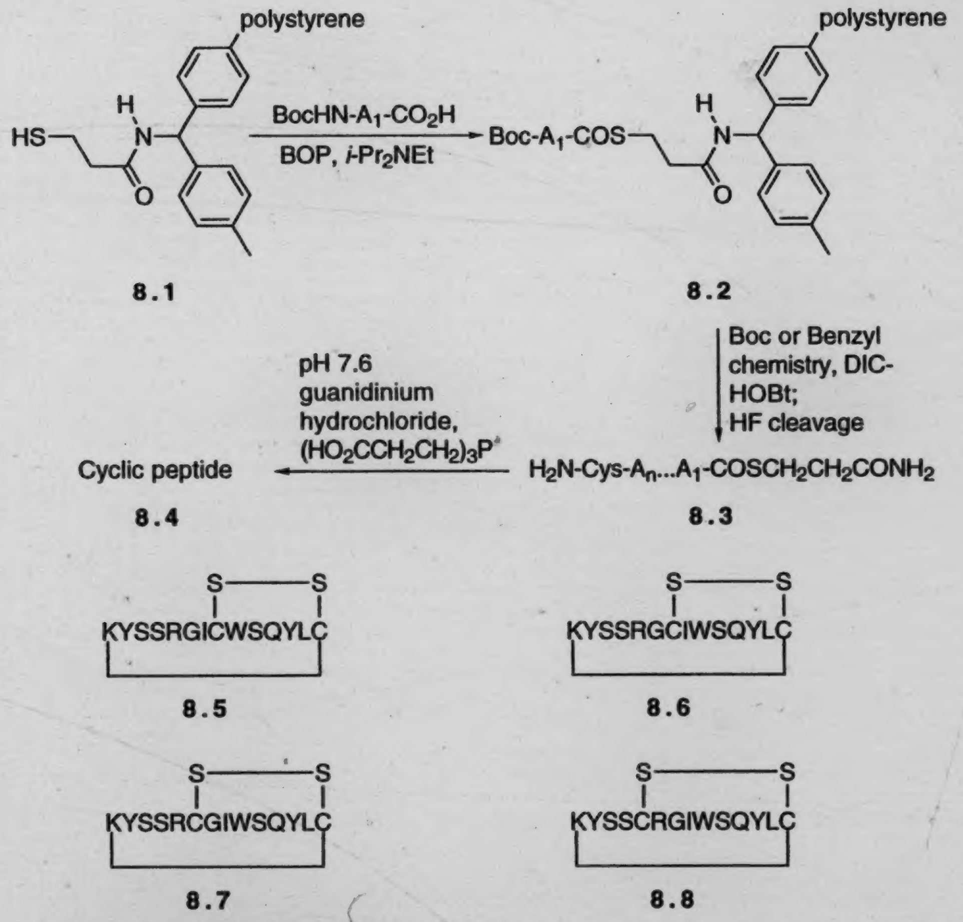

TrS
H
H
N
N
O
O
polystyrene
7.1
H2N-LYRA
N-H
R
S
O
polystyrene
7.2
HF
H2N
O
RANK-OH
HS
7.4
H2N-LYRA
H
N
O
S
O
N
H
O
NH2
R
7.3
pH 7, 6 M guanidinium hydrochloride, BnSH, PhSH
H2N-LYRA
H
N
O
N
H
SH
RANK-OH
O
R
7.5

Scheme 7

It was found[18] that ligation occurred when X represented any of the 20 natural amino acids, although when X = Pro, Ile, and Val ligation was slow, with the ligation-acyl transfer sequence being less than 75% complete after 48 h. Interestingly, when X = His or Cys, reaction is as fast as with Gly, the least hindered amino acid. It is possible that the side chains of His and Cys participate in catalysis of the rate-limiting step – the transthioesterification. Racemization of amino acid X at the X-Cys ligation site was examined for the case of X = His, and found to be less than 2%.[18]

The approach of Scheme 7 has been used[27] to produce cyclic peptides by native chemical ligation, as summarized in Scheme 8. Linear peptides were made by solid phase synthesis, using the resin **8.1**. After attachment of the initial amino acid, standard stepwise assembly, using Boc- and benzyl-chemistry, followed by detachment from the resin, gave the required linear peptides. When these were subjected to standard conditions for native chemical ligation, intramolecular transthioesterification occurred, and then acyl transfer produced the final cyclic peptide. While internal Cys residues also underwent transthioesterification with the *C*-terminal thioester (see **8.3**), formation of such thiolactones is reversible and they are eventually converted into the productive *N*-terminal thiolactone that undergoes the *S* → *N* acyl transfer. The initial products were oxidized to cyclic disulfides using DMSO in an aqueous buffer at pH 5-6. In this way, cyclic peptides **8.5**-**8.8** were prepared.

Cyclizations can also be effected[27] before detachment


polystyrene
H
HS
N
O
BocHN-A₁-CO₂H
BOP, i-Pr₂NEt
Boc-A₁-COS
polystyrene
H
N
O
8.1
8.2
Boc or Benzyl chemistry, DIC-HOBt;
HF cleavage
pH 7.6
guanidinium hydrochloride,
(HO₂CCH₂CH₂)₃P
Cyclic peptide
H₂N-Cys-Aₙ...A₁-COSCH₂CH₂CONH₂
8.4
8.3
S
S
KYSSRGICWSQYLC
8.5
S
S
KYSSRGCIWSQYLC
8.6
S
S
KYSSRCGIWSQYLC
8.7
S
S
KYSSCRGIWSQYLC
8.8

BOP = benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
DIC = diisopropylcarbodiimide
HOBt = 1-hydroxybenzotriazole

Scheme 8

from the resin, but in this case, a different thioester linker and resin were used. After deprotection of the resin-bound peptide, intramolecular transthioesterification served to release the peptide from the resin and set the stage for acyl transfer.

The peptide H_2N-CGGGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEP-GG-COSR, made by solid phase peptide synthesis, using Boc-chemistry, was cyclized[28] to give a catenane; this was possible because the peptide spontaneously folds and dimerizes *faster* than ligation. In this case, of course, the Cys residue of each component of the initial dimer ligates with the thioester terminus of the other component; only in this way can a catenane be formed.

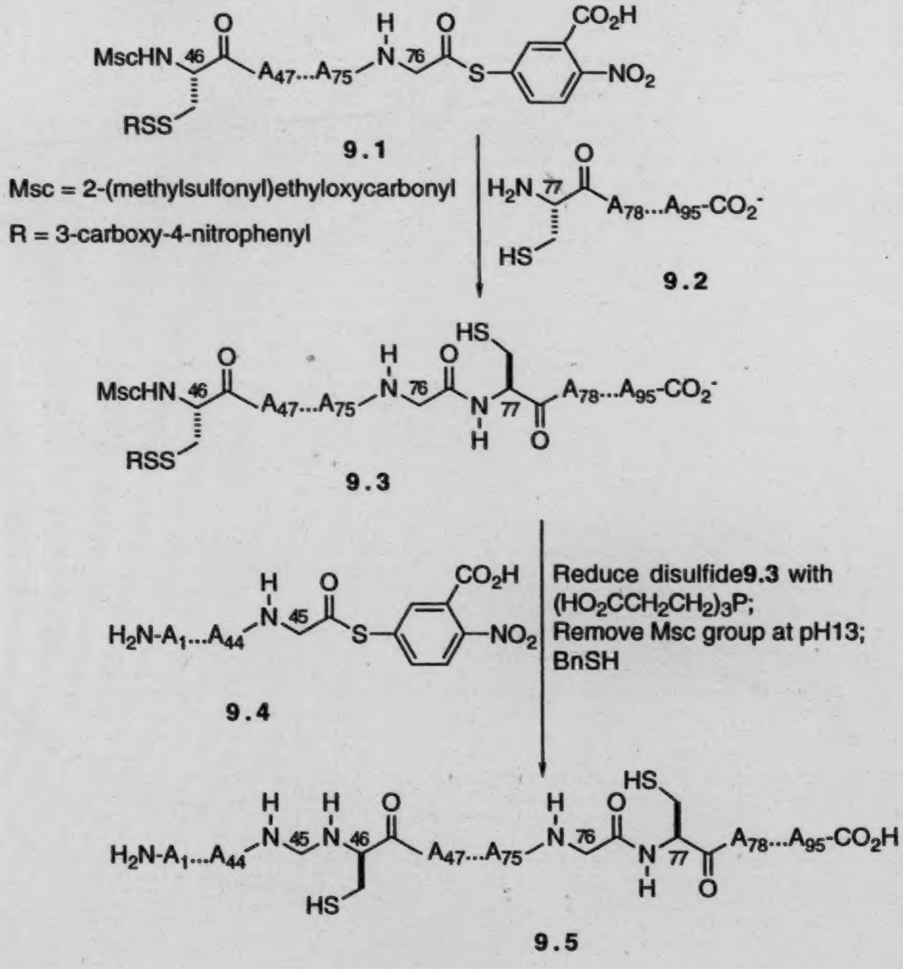
A number of other cyclic peptides[29,30,31,32] have been made by the general technique of subjecting $H_2N\text{-Cys-}A_1\ldots A_n\text{-}$ COSR to conditions for native chemical ligation.

Biosynthetic methods[33,34] have also been used to generate thioesters, but these routes are beyond the scope of this review.

Multiple native chemical ligations and ligations on a solid phase

Multiple native chemical ligations have been explored,[11] and in an early test of this process, an analog of a natural protein containing 95 amino acid residues was made. To accomplish this, two pairs of residues were changed from the wild type: Ser^{45} and Ser^{76} were both changed to Gly to improve the kinetics of the ligation steps, and Lys^{46} and Arg^{77} were both changed to Cys, as the presence of Cys is essential for the ligation. Three subunits **9.1**, **9.2**, and **9.4** were made by solid phase peptide synthesis.

In the first ligation and acyl transfer (**9.1** + **9.2** → **9.3**) the Cys terminus of **9.1** was protected on nitrogen by a base labile group, and the thiol was protected as a disulfide


9.1
Msc = 2-(methylsulfonyl)ethyloxycarbonyl
R = 3-carboxy-4-nitrophenyl
9.2
9.3
Reduce disulfide9.3 with
(HO2CCH2CH2)3P;
Remove Msc group at pH13;
BnSH
9.4
9.5

Scheme 9

Hence only the desired ligation took place. In the final ligation BnSH was included in the reaction mixture to reverse unproductive reaction of the thiol of Cys^{77}. The successful synthesis of **9.5** suggested that multiple ligations could be used to make proteins with well in excess of 100 amino acid residues.

The 124-amino acid polypeptide chain of human secretory phospholipase A_2 (hsPLA$_2$) was synthesized as well as an analog

H$_2$N-N^{1}LVNFH(Dnp)RMIKLTTGKEAALSYGFYGCH^{27}(Dnp)-SR

10.1

MscHN-C^{28}GVGGRGSPKDATDRCCVTH(Dnp)DCCYKRLEKRG^{58}-SR

10.2

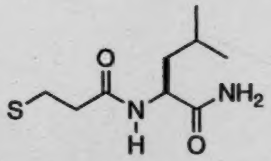
MscHN-C^{59}GTKFLSYKFSNSGSRITCAKQDSCRSQL^{87}-SR

10.3

H$_2$N-C^{88}ECDKAAATCFARNKTTYNKKYQYYSNKH(Dnp)CRGSTPRC^{124}-OH

10.4

SR =



Msc = 2-(methylsulfonyl)ethyloxycarbonyl

Dnp = 2,4-dinitrophenyl

Scheme 10

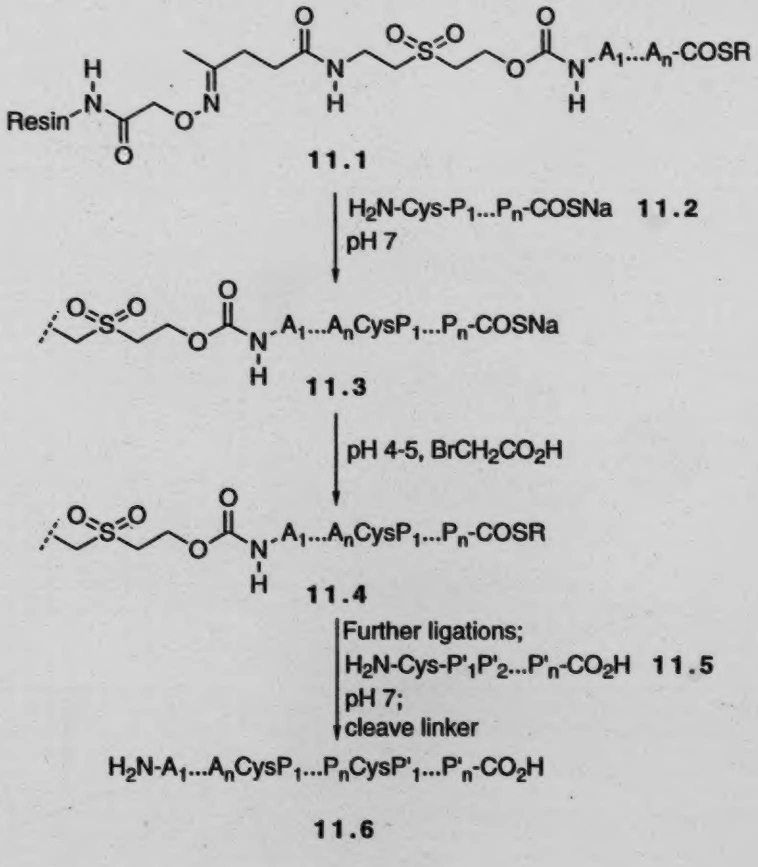
in which His^{47} was replaced by the isosteric β-thienylalanine (Bta).[18]

To make hsPLA$_2$, the four subunits **10.1-10.4** were prepared by solid phase methods using, in the case of **10.1-10.3**, the special resin (see Scheme 7) for producing thioesters. Subunits **10.3** and **10.4** were coupled and required 24 h at 37 °C for 90% ligation. After removal of the Msc group the resulting (59-124)-peptide was coupled with **10.2**, and with an analog (not show in Scheme 10) in which His^{47} was replaced by Bta^{47}. These reactions with **10.2** involve Gly-Cys ligations and, accordingly, were complete in only 6 h. Again, after removal of the Msc group, the resulting materials were coupled with **10.1**. The reactions, which generate a His(Dnp)-Cys ligation site were complete in 9 h. The Dnp groups were probably removed during the ligations, but this is not clearly stated in the publication.[18]

Typically, for native chemical ligation, each ligation affords the desired product in yields of 40-60% and, in order to improve the efficiency of multiple native chemical ligations by avoiding handling losses (HPLC purifications), ligations on a solid support have been studied[35,36,37] (Schemes 11[35] and 13[36]).

In one approach[35] the polypeptide can be assembled in either the *N* → *C* or the *C* → *N* direction. For *N* → *C* assembly, the middle segments were used in the form H_2N-Cys-(peptide)$^{\alpha}$COSNa because the thiocarboxylate – unlike the corresponding ester – is unreactive under ligation conditions so that the *N*-terminal Cys does not react with the *C*-terminal thiocarboxylate. For *C* → *N* assembly, the middle segments were used in the form H_2N-Cys(Acm)-(peptide)$^{\alpha}$COSR, the sulfur protecting group at the *N*-terminal Cys preventing premature ligation with the $^{\alpha}$COSR terminus. The segments were synthesized by standard, or previously-developed, methods on $^{\alpha}$COSH, $^{\alpha}$COSR, or PAM resins.

For construction in the *N* → *C* direction the *N*-terminal segment was attached to a water-compatible support (based on cellulose) by a cleavable linker (see **11.1**). The next peptide segment (**11.2**) was added under native chemical ligation conditions (pH 7, 1% PhSH). Once ligation was complete, the pH was lowered to 4-5, and the terminal thiocarboxylate was allowed to react with $BrCH_2CO_2H$ so as to form a terminal thioester (**11.3** → **11.4**). The pH was then returned to 7 and the polymer-bound peptide thioester was then ready for the next ligation. This cycle was repeated and, after ligation of the final peptide segment the linker to the support was cleaved by brief treatment with NaOH, freeing the full-length peptide **11.6**.

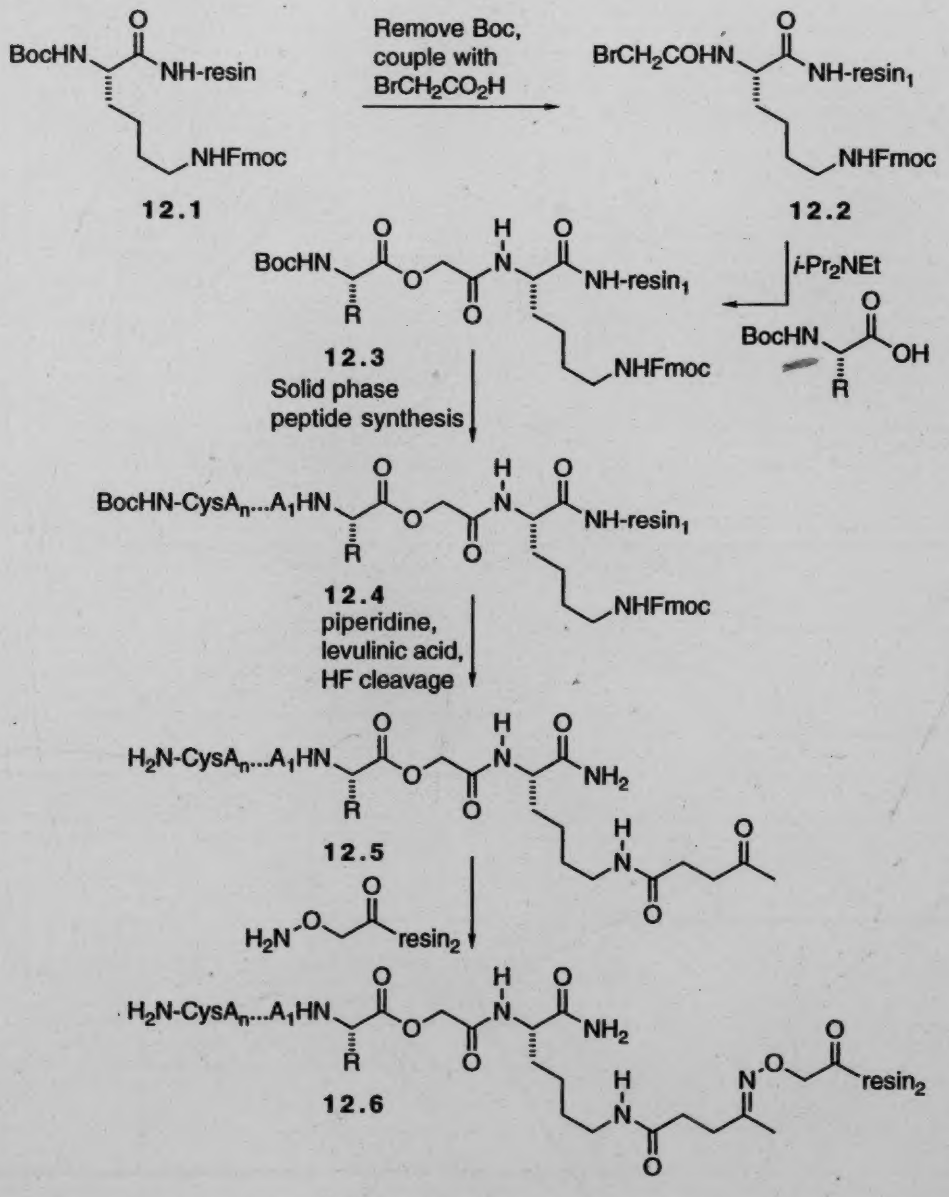

Resin
A1...An-COSR
11.1
H2N-Cys-P1...Pn-COSNa 11.2
pH 7
A1...AnCysP1...Pn-COSNa
11.3
pH 4-5, BrCH2CO2H
A1...AnCysP1...Pn-COSR
11.4
Further ligations;
H2N-Cys-P'1P'2...P'n-CO2H 11.5
pH 7;
cleave linker
H2N-A1...AnCysP1...PnCysP'1...P'n-CO2H
11.6

Scheme 11

To illustrate the efficacy of the technique, several polypeptides were made, each requiring two sequential ligation steps. First, three arbitrary peptide segments were coupled, these being H$_2$N-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKH$^{\alpha}$COSR at the *N*-terminus, H$_2$N-CGFRVREFGDNTAV$^{\alpha}$COSNa for the middle segment, and H$_2$N-CADPSEEWVQKYVSDLELSA-OH as the *C*-terminus, so as to produce a 68-residue peptide.

In the second example a 74-residue protein belonging to the human complement system[38] was made, using the three segments H$_2$N-TLQKKIEEAAKYKHSVVKK$^{\alpha}$COSR (*N*-terminus of 20 residues), H$_2$N-CCYDGACVNNDETCEQRAARISLGPK$^{\alpha}$COSNa (middle segment of 26 residues), and H$_2$N-CIKAFTECCVVASQLRANISHKDMQLGR-OH (*C*-terminus of 28 residues). Finally, the 115-residue polypeptide of the protein macrophage migration inhibitor,[39] a mediator of inflammatory response, was constructed from the three segments H$_2$N-MPMFIVNTNVPRASVPDGFLSELTQQLAQATGKPPQYIAVHVVPDQLMAFGGSSEPCAL$^{\alpha}$COSR (*N*-terminus of 59 residues), H$_2$N-CSLHSIGKIGGAQNRSYSKLL$^{\alpha}$COSNa (middle segment of 21 residues), and H$_2$N-CGLLAERLRISPDRVYINYYDMNAASVGWNNSTFA-OH (*C*-terminus of 35 residues).

Construction in the *C* → *N* direction is summarized in Scheme 12, where resin$_1$ is a polystyrene-based resin and Resin$_2$ is based on cellulose. The *C*-terminal peptide is built up on the first resin (Scheme 12), using the linker shown in **12.3**. This linking system, part of which is based on the carboxyamidomethyl protecting group for carboxylic acids, is compatible with Boc- and Fmoc-based solid phase peptide synthesis, and can be detached at the end of the synthesis by mild base treatment. The linker also allows a water-compatible support to be joined (see **12.4** → **12.5** → **12.6**) to the lower Fmoc-protected arm, and the first resin can then be removed.


BocHN
NH-resin
NHFmoc
12.1
Remove Boc, couple with $BrCH_2CO_2H$
$BrCH_2COHN$
$NH-resin_1$
NHFmoc
12.2
$i-Pr_2NEt$
BocHN
OH
R
12.3
Solid phase peptide synthesis
$BocHN-CysA_n...A_1HN$
12.4
piperidine, levulinic acid, HF cleavage
$H_2N-CysA_n...A_1HN$
NH_2
12.5
H_2N
$resin_2$
12.6

Scheme 12

The second peptide segment, bearing a protected *N*-terminal Cys and a *C*-terminal thioester, is then attached under native chemical ligation conditions (pH 7, 1% PhSH) to the first segment (i.e. to **12.6**), which is still attached to the water-compatible polymer (resin 2). The *N*-terminal Cys of each of these middle segments was protected on sulfur with an Acm group ($CH_2NHCOMe$). This group is stable to CF_3CO_2H, HF, nucleophiles (including thiols), and is readily removed with $Hg(OAc)_2$.

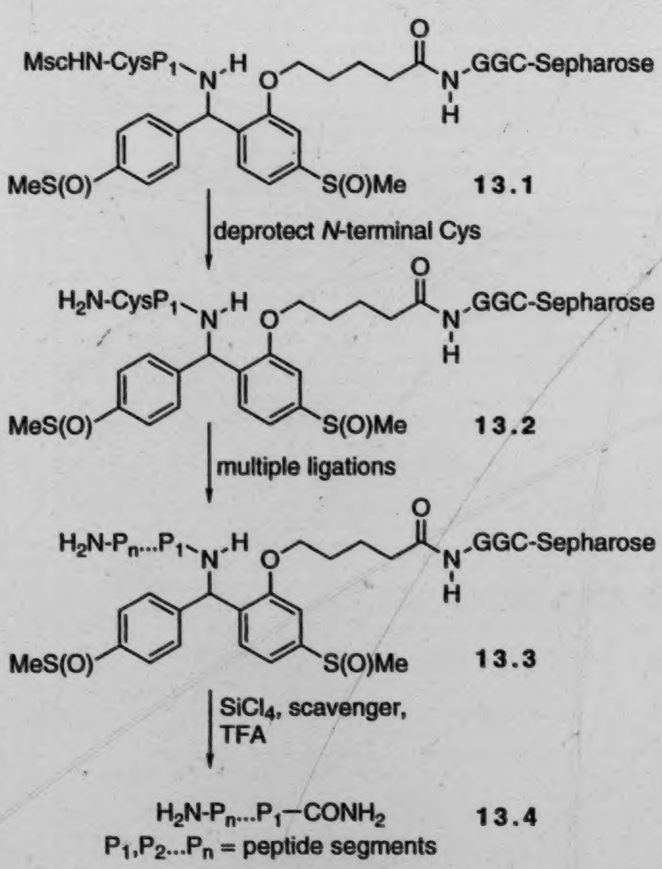
After ligation, the *N*-terminal Cys is deprotected, and the material is ready for a second ligation. This cycle is repeated and, after the final segment has been attached, the linker to the water-compatible polymer support is cleaved at pH 12-14 [to cleave the carboxyamidomethyl unit, $OCH_2C(O)NH$], releasing the unprotected polypeptide.

The *C* → *N* construction was tested by making the 27-residue peptide H_2N-ALTKYGFYGCYGRLEEKGCADRKNILA-OH from the appropriate segments H_2N-CADRKNILA-*O*-linker (cf. **12.4**), H_2N-C(Acm)YGRLEEKG$^{\alpha}$COSR, and H_2N-ALTKYGFYG$^{\alpha}$COSR.

The 118-residue protein human group V secretory phospholipase A_2 (GV-PLA_2)[40] was made to illustrate synthesis of a protein by solid phase *C* → *N* construction. The sequence was divided into four segments at suitably located Cys residues, using three native chemical ligations. The segments ranged in length from 25 to 33 amino acids and consisted of the sequences GV-PLA_2(88-118), GV-PLA_2(59-87), GV-PLA_2(26-58), and GV-PLA_2(1-25). All peptide solutions ranged in concentrations from 11 to 14 mM, but evidence was obtained that using higher concentrations (27 - 50 mM, depending on the solubility of the segment) and shorter reaction times would allow the synthesis to be completed more quickly. The first ligation – that between Leu^{87} and Cys^{88} – was slower than the others, which were Gly-Cys ligations.

The method has been used to couple up to seven unprotected peptide segments.[41]

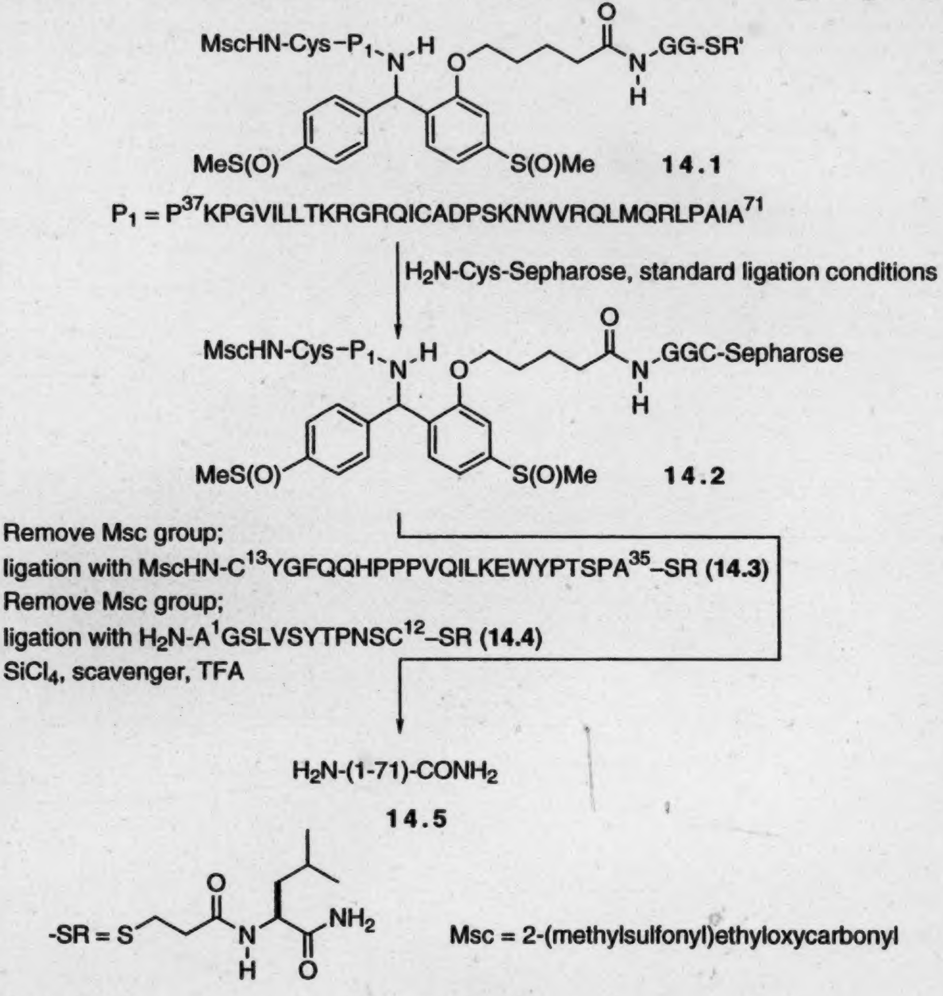
In related work,[36] the first peptide segment was connected to the solid support via the complicated linking


MscHN-CysP$_1$
N
H
O
O
N
H
GGC-Sepharose
MeS(O)
S(O)Me
13.1
deprotect N-terminal Cys
H$_2$N-CysP$_1$
13.2
multiple ligations
H$_2$N-P$_n$...P$_1$
13.3
SiCl$_4$, scavenger, TFA
H$_2$N-P$_n$...P$_1$–CONH$_2$
13.4
P$_1$,P$_2$...P$_n$ = peptide segments

Scheme 13

unit shown in **13.1**. The best support was found to be Sepahrose (4% cross-linked agarose functionalized with primary amino groups). The actual attachment was by a native chemical ligation – hence the linking amino acid is a Cys residue attached to the resin (see **13.1**). The system is stable to the conditions used in Boc-chemistry and, of course, in native chemical ligation; cleavage of the final polypeptide from the linker is achieved by reduction ($SiCl_4$) of the sulfoxide groups, followed by exposure to CF_3CO_2H. The *N*-terminal Cys residues of all the middle segments (see Scheme 13, P_1, P_2, P_3...P_{n-1}) were protected either on *N* as 2-(methylsulfonyl)ethyloxycarbonyl derivatives [Msc] or on *S* as acetamidomethyl derivatives ($SCH_2NHCOMe$). The Msc group is removed by brief exposure to pH 13, and the Acm group by treatment with $Hg(OAc)_2$ at pH 4. The approach summarized in Scheme 13 affords a peptide with a *C*-terminal amide (see **13.4**).

In order to demonstrate the use of the solid phase method in protein synthesis a 71-amino acid chemokine [vMIP-I] was synthesized (Scheme 14).[36] This target was chosen because it contains all 20 natural amino acids. The route is summarized in Scheme 14. The *C*-terminal segment **14.1** was made on a thioester resin (see **14.1**; the group R in **14.1** was not specified in the original publication), the *N*-terminus of this segment being protected with an Msc group. This unit was attached to a Cys-Sepharose support using the standard conditions for native chemical ligation. The Msc group was


MscHN-Cys–P1 N H O O N GG-SR' H
MeS(O) S(O)Me
14.1
P1 = P37KPGVILLTKRGRQICADPSKNWVRQLMQRLPAIA71
H2N-Cys-Sepharose, standard ligation conditions
MscHN-Cys–P1 N H O O N GGC-Sepharose H
MeS(O) S(O)Me
14.2
Remove Msc group;
ligation with MscHN-C13YGFQQHPPPVQILKEWYPTSPA35–SR (14.3)
Remove Msc group;
ligation with H2N-A1GSLVSYTPNSC12–SR (14.4)
SiCl4, scavenger, TFA
H2N-(1-71)-CONH2
14.5
-SR = S O N H NH2 O
Msc = 2-(methylsulfonyl)ethyloxycarbonyl

Scheme 14

removed and the resulting peptide was ligated with the Msc-(13-35)COSR segment **14.3**. Finally, after deprotection of the terminal Cys, a third ligation was done with the H_2N-(1-12)COSR segment **14.4**. The resulting resin-bound 71-amino acid peptide was treated with $SiCl_4$ in the presence of several scavengers (thioanisole, *m*-cresol, ethanedithiol) in CF_3CO_2H to release the protein **14.5**.

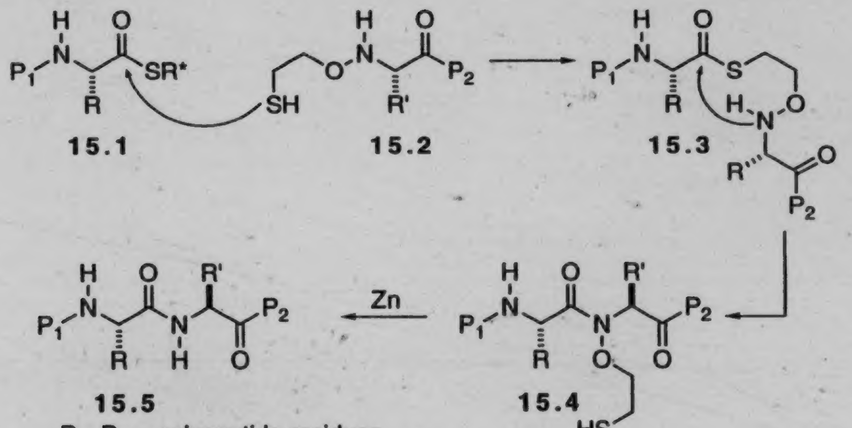
A second synthesis[36] was carried out using Cys(S^{β}Acm) on the side chain thiol of the *N*-terminal Cys residues, simply to show that a choice of protection methods is available for Cys. The peptide thioesters and H_2N-C^{36}(S^{β}Acm)PKPGVILLTKR-GRQICADPSKNWVRQLMQRLPAIA^{71}-SR, H_2N-C^{13}(S^{β}Acm)YGFQQHPPPVQILKE-WYPTSPA^{35}-SR and H_2N-A^{1}GSLVSYTPNSC^{12}-SR (**14.4**) were assembled as before, except that the Acm (acetamidomethyl, $SCH_2NHCOMe$) groups were removed by treatment with $Hg(OAc)_2$ at pH 4. This procedure gave the protein **14.5** in quantitative yield and of similar purity to the synthesis using Msc protecting groups.

Extension of the ligation site beyond cysteine

Cys is an uncommon amino acid, comprising only 1.7% of the residues in proteins[19] (only Trp, Met, and His appear less frequently[42]) and so extension of the ligation site beyond Cys is an important aim.

(a) Glycine left at the ligation site instead of cysteine

Some extension beyond Cys of the suitable residues at the ligation site was made by use of an oxyethyl linker.[43] The peptide α-thioester **15.1** reacts with the N^{α}(oxyethanethiol) peptide **15.2** to form the ligation product **15.3**. This rearranges through a six-membered ring to the amide-linked product **15.4**. The N^{α}(oxyethanethiol) subunit is stable to HF cleavage conditions but is removed by reduction with Zn (**15.4** → **15.5**).

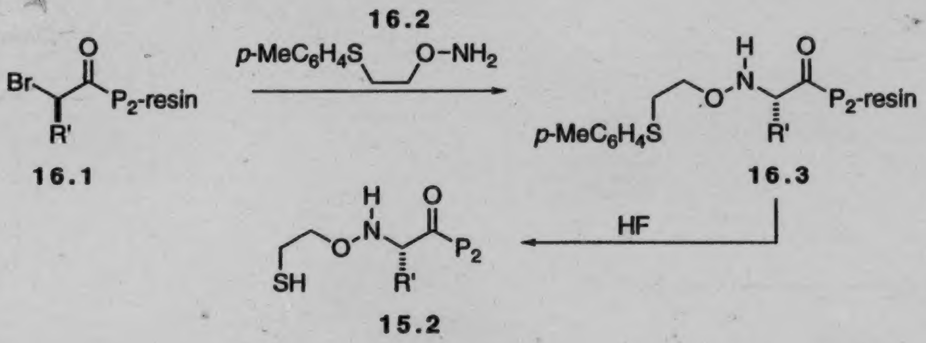

15.1
15.2
15.3
15.4
15.5
SR*
SH
HS
Zn

P_1, P_2 = polypeptide residues

Scheme 15

In implementing this approach[43] the peptide thioesters were generated from the corresponding thioacids, themselves made by established solid phase methods. Alkylation of the thioacids with BnBr or reaction with 5,5′-dithiobis(2-nitrobenzoic acid) gave the thioesters, both types being equally suitable for the subsequent ligation, with the benzyl thioesters reacting more slowly.

The oxyethanethiol peptides **15.2** were made by bromide


16.2
16.1
16.3
15.2
P2-resin
p-MeC6H4S
O-NH2
HF

Scheme 16

displacement (with stereochemical inversion) along the lines summarized in Scheme 16. The starting bromo peptide **16.1** was made by acylation of the *N*-terminus using the symmetrical anhydride $(BrCHRCO)_2O$, and the halogen was then displaced with reagent **16.2**. Cleavage from the resin and deprotection, both done with HF, then gave peptide segment **15.2**.

Scheme 15 summarizes the principle of the method; a number of particular cases were examined, as summarized in Scheme 17.

	Thioester 15.1	**Derivatized peptide 15.2**	**Yield of 15.4**
1	H_2N-LYRAG-SNB	HN^{α}(X)-GRNTATIMMQRGNFR$^{\alpha}CONH_2$	75%
2	H_2N-LYRAF-SBn	HN^{α}(X)-GRNTATIMMQRGNFR$^{\alpha}CONH_2$	64%
3	H_2N-LYRAG-SBn	HN^{α}(X)-AARHTVHQRHLHG-OH	69%
4	H_2N-LYRAF-SBn	HN^{α}(X)-AARHTVHQRHLHG-OH	0%

SNB = 3-carboxy-4-nitrophenyl thioester, X = $HSCH_2CH_2O$

Scheme 17

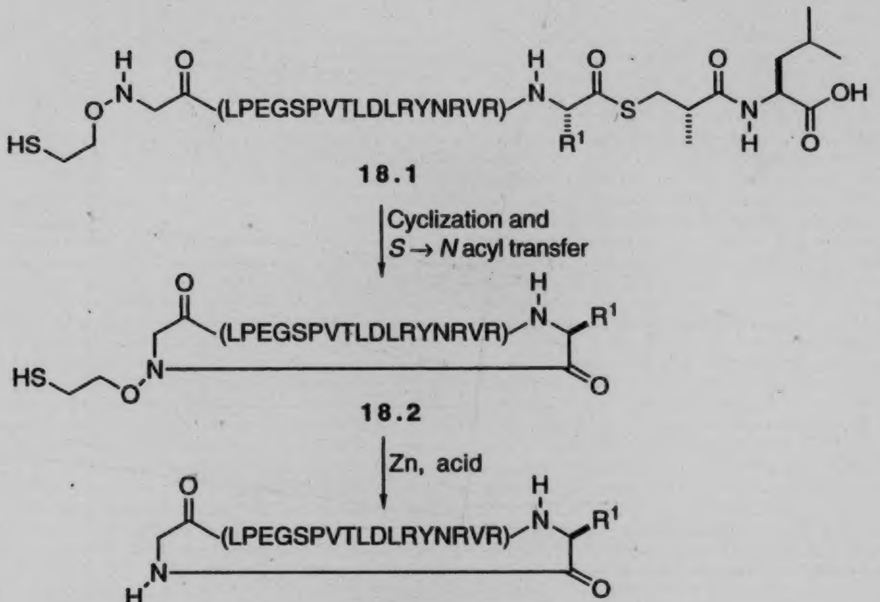
In the first ligation an overnight reaction period at 25 °C gave the rearranged product (cf. **15.4**) in 75% yield, and Zn reduction removed the auxiliary. The second ligation involves the thioester of a more hindered *C*-terminal amino acid (Phe versus Gly); reaction was slowed compared with he first ligation, but heating to 37 °C or lowering the pH to 4 after the initial ligation accelerated the acyl transfer. The rearrangement (cf. **15.3** → **15.4**) was also retarded, and the intermediate corresponding to **15.3** could be isolated. After 10 h at 37 °C at pH 4.5 rearrangement of the initial ligation product was complete.

Ligation #3 served to reveal the influence of steric hindrance adjacent to the *N*-modified amino acid **15.2** (Ala versus Gly). The rate was similar to that of ligation #2, and was also enhanced by heating at 37 °C. Again the initial ligation product could be isolated, but its rearrangement was not accelerated by lowering the pH to 4.5. After 2 days at 37 °C rearrangement was complete.

When sterically hindered amino acids are present on both sides of the intended ligation site – Phe versus Gly at the thioester terminus and Ala versus Gly adjacent to the *N*-modified amino acid – as in the last example, the initial ligation did occur, but there was no evidence for the rearrangement (cf. **15.3** → **15.4**). Evidently, the presence of side chains on both sides of the ligation site provided too much steric hindrance for rearrangement via a six-membered intermediate.

These results suggest that the method will prove suitable for ligations in which one of the amino acids flanking the ligation site is Gly, and this amino acid can be part of the thioester unit **15.1** or the other segment **15.2**; β-branched amino acids in both **15.1** and **15.2** cause the method to fail. The rearrangement in the present approach involves a six-membered transition state, and was found to be appreciably slower than the corresponding rearrangement of the original native chemical ligation, which involves a five-membered transition state.

The procedure has also been applied to the synthesis of



Scheme 18

cyclic peptides (Scheme 18).[44]

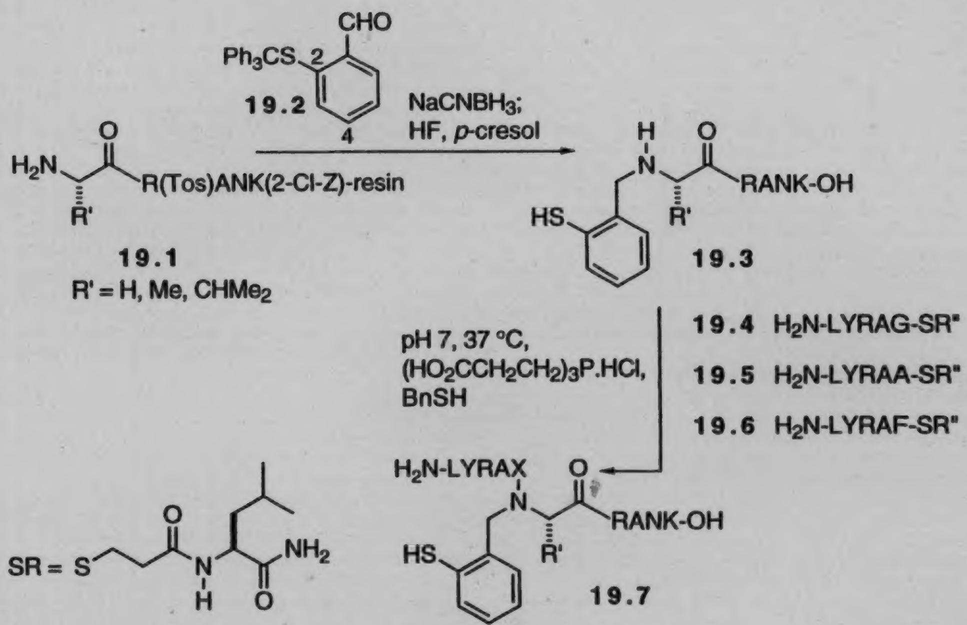
The linear peptides **18.1** (R^1 = H, Me, $PhCH_2$) were prepared, using established methods,[43] and subjected to conditions for ligation and acyl transfer (6M guanidine hydrochloride, pH 7.5 buffer, PhSH). Reduction of the resulting cyclized products **18.2** served to remove the $HSCH_2CH_2O$-group and gave the desired cyclic peptides **18.3** with a native backbone structure.

The rate of ligation depended on the nature of the amino acid residues being linked: for a Gly-Gly ligation (**18.1**, R^1 = H) the process was complete in 16 h. For Gly-Ala (**18.1**, R^1 = Me) and Gly-Phe (**18.1**, R^1 = CH_2Ph) ligations two products – presumed to correspond to the amide and the intermediate thioester – were detected after 12 h. Only lowering the pH from 7.5 to 4.5 was effective in driving the reaction to completion (in 48 h).

For **18.1** ($R^1 = PhCH_2$) no detectable (i.e. <5%) racemization was observed. This was established by using also D-Phe in the synthesis and examining the product from the natural series for the presence of cyclic peptide containing the unnatural phenylalanine diastereoisomer.

(b) Attempts to develop a general auxiliary that allows any amino acid at the ligation site[45]

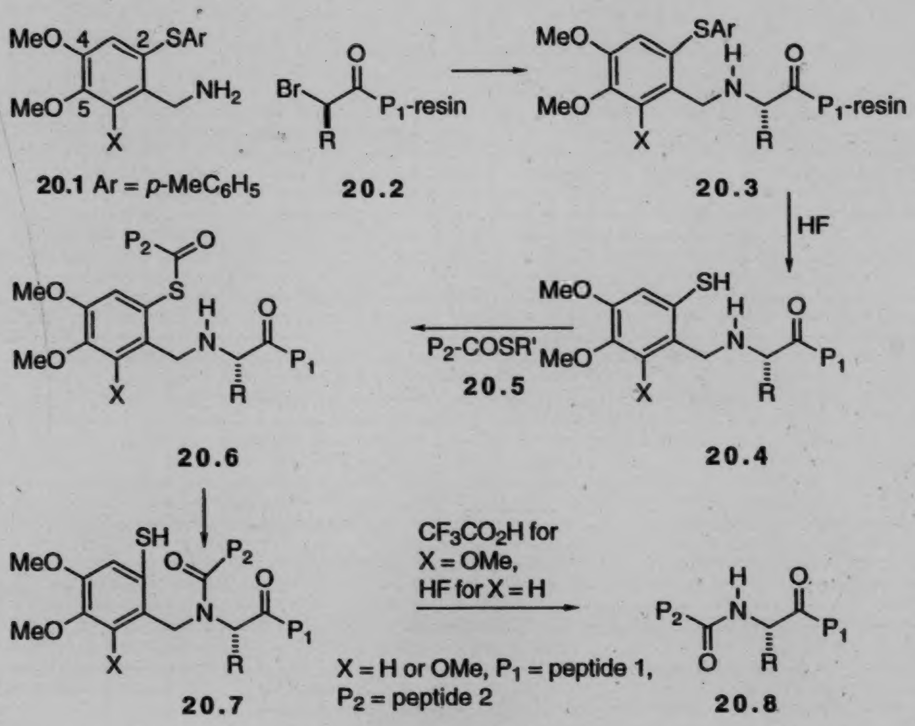
The resin bound protected peptide **19.1** was treated with aldehyde **19.2**, and the resulting imine was reduced. Cleavage from the resin gave pentapeptide **19.3**. The value of R′ was varied (R′ = H, Me, $CHMe_2$) in different experiments, and the individual pentapeptides **19.3** were then ligated with (depending on the case) **19.4**, **19.5**, or **19.6**. Half-lives were measured for production of the decapeptides **19.7**, and varied from 0.5 h to 48 h. In the case of **19.3** (R′ = $CHMe_2$) ligation with **19.4** (other thioesters were not tested) reaction was less than 5% complete after 24 h. The experiments established that ligations involving Gly at either side of the ligation site go to completion in under 24 h, provided β-branched amino acids (e.g. Val) or proline are absent from the other side. In this work the auxiliary was not removed from the coupled peptide. When a nitro group was placed at C(4) in **19.2** the peptide corresponding to **19.3** did not undergo ligations, as the sulfur was no longer


Ph3CS
CHO
2
4
19.2
NaCNBH3;
HF, p-cresol
H2N
R(Tos)ANK(2-Cl-Z)-resin
R'
19.1
R' = H, Me, CHMe2
HS
RANK-OH
19.3
pH 7, 37 °C,
(HO2CCH2CH2)3P.HCl,
BnSH
19.4 H2N-LYRAG-SR"
19.5 H2N-LYRAA-SR"
19.6 H2N-LYRAF-SR"
H2N-LYRAX
19.7
SR =
NH2

Scheme 19

nucleophilic enough.

A more advanced version of the approach summarized in Scheme 19 is shown in Scheme 20.[46] In this case, a di- or trimethoxybenzyl unit was used. This unit bears a protected sulfur *ortho* to the benzylic carbon (see **20.1**). Bromide displacement, with stereochemical inversion (**20.1** → **20.3**), gives a resin-bound peptide carrying the specially derivatized *N*-terminal amino acid. No epimerization (<2%) of the *N*-terminal amino acid was observed with small model peptides using this preparative method. Deprotection of the sulfur and cleavage from the resin (both done with HF), ligation with peptide thioesters **20.5**, and acyl transfer (**20.3** → **20.4** → **20.6** → **20.7**) sets the stage for removal of the auxiliary and release of the native peptide. When X = H,



MeO
MeO
4
2
5
SAr
NH₂
X
20.1 Ar = p-MeC₆H₅
Br
R
O
P₁-resin
20.2
H
N
20.3
HF
SH
20.4
P₂-COSR'
20.5
P₂
S
P₁
20.6
20.7
CF₃CO₂H for
X = OMe,
HF for X = H
X = H or OMe, P₁ = peptide 1,
P₂ = peptide 2
20.8

Scheme 20

the auxiliary is removed (**20.7** → **20.8**) with HF but, for the trimethoxy series (X = OMe) CF_3CO_2H containing *i*-Pr_3SiH is adequate. The methoxy group at C(5) increases the efficiency of the thioester exchange – which is usually, the rate-limiting step.

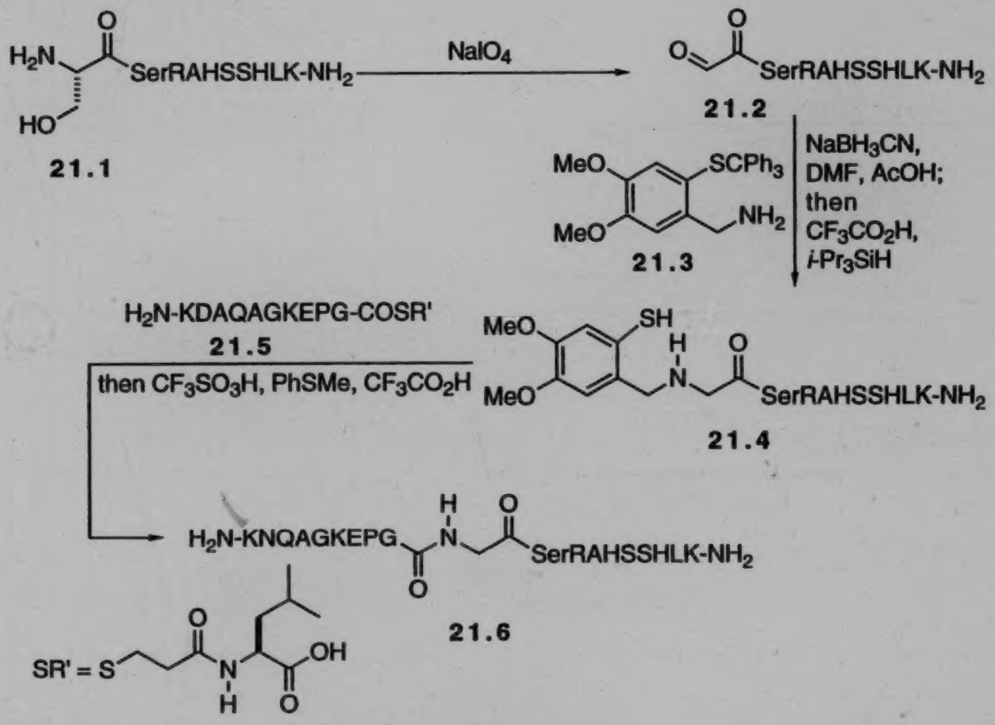
In order to demonstrate the effectiveness of the methodology, the 62-amino acid SH3 domain of α-spectrin was synthesized[46] by ligation at a Lys-Gly site. This site was chosen as representing a typical ligation site in a protein, it being already established[18,43,47] that the rate of ligation is sensitive to the nature of the *C*-terminal amino acid thioester: the rate is high with Gly and His thioesters and low for thioesters of β-branched amino acids. These extreme cases are not regarded[46] as being representative of the ligation properties of the majority of amino acids such as Leu and Lys.

In order to synthesize the protein, the segment α-spectrin[1-27] thioester, i.e. H_2N- MDETGKELVLALYDYQEKSPREVT-MKK-SC_6H_5 and Dmb-α-spectrin[28-62], i.e. HN(dimethoxy auxiliary)-GDILTLLNSTNKDWWKVEVNDRQGFVPAAYVKKLD-OH (cf. **20.4**), were added to an aqueous solution of 6 M guanidine hydrochloride containing a phosphate buffer initially at pH 8.5. The mixture had pH 7 after addition of the peptides, and the coupled product was isolated in 66% yield.

The scope of the procedure was investigated further by attempting ligations at three additional sites in α-spectrin-like peptides, involving ligation of a Gly-thioester and a derivatized *N*-terminal Gly, a Gly-thioester and a derivatized *N*-terminal Ala, and an Ala-thioester and a derivatized *N*-terminal Ala. For these experiments segments of similar length to those used initially in the synthesis of the SH3 domain of α-spectrin were again used. The Gly-SC_6H_5/(Auxiliary)Gly coupling (with the dimethoxy auxiliary) was rapid (half-life 0.2 h); the Gly-SC_6H_5/(Auxiliary)Ala coupling had a half-life of 5 h (dimethoxy auxiliary); no reaction was observed on attempting a Ala-SC_6H_5/(Auxiliary)Ala ligation. Lys-SC_6H_5/(Auxiliary)Gly couplings with either the di- or trimethoxy auxiliaries had $t_{1/2}$ of 2 h. The fact that Lys-SC_6H_5/(Auxiliary)Gly couplings are slower than Gly-

SC_6H_5/(Auxiliary)Gly couplings is consistent with ester exchange being the rate-limiting step, while the large rate decrease between derivatized Gly ligations and derivatized Ala ligations with Gly thioesters suggests that the rate-limiting step is acyl transfer in these two cases. The experiments with **20.1** (X = H or OMe) suggest that these auxiliaries are very useful for ligations at Gly-Gly sites.

The auxiliary of type **20.1** (X = H) can be introduced in a different way[48] from that shown in Scheme 20. Periodate oxidation of peptide segment **21.1** (Scheme 21) carrying an *N*-terminal serine affords the corresponding segment with an *N*-terminal glyoxyloyl group (**21.2**), and this can be subjected to reductive amination with amine **21.3**. Deprotection of the



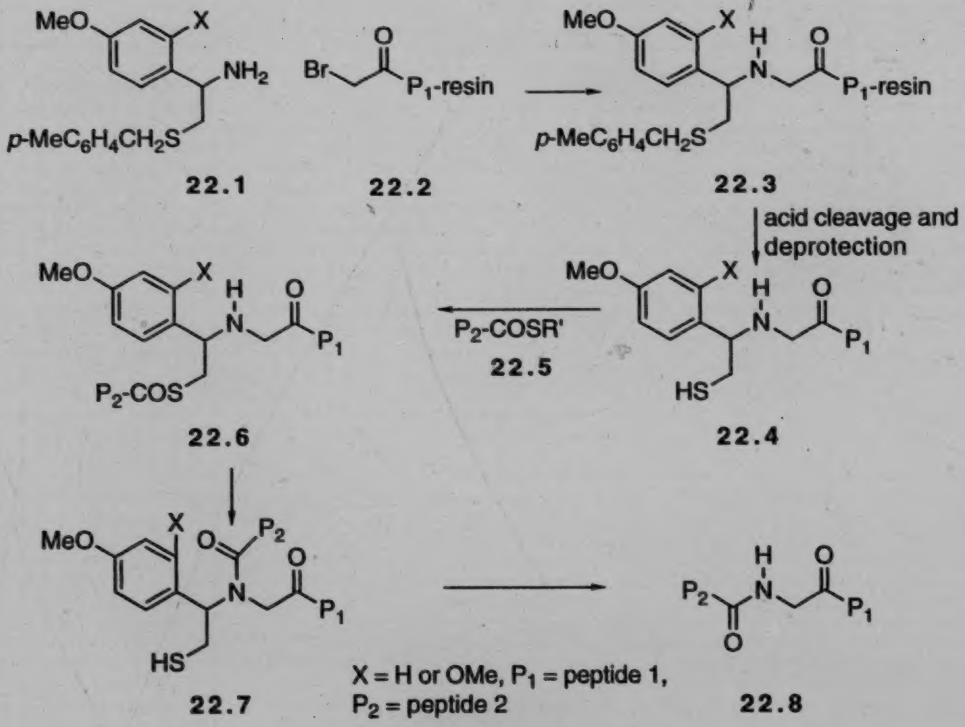
SerRAHSSHLK-NH2
NaIO4
21.1
21.2
21.3
21.4
21.5
21.6
NaBH3CN, DMF, AcOH; then CF3CO2H, i-Pr3SiH
H2N-KDAQAGKEPG-COSR'
then CF3SO3H, PhSMe, CF3CO2H
H2N-KNQAGKEPG
SR' =

Scheme 21

sulfur (CF_3CO_2H, *i*-Pr_2SiH) then affords a peptide with an *N*-terminus correctly constituted for ligation with a peptide thioester (**21.5**) and acyl transfer under standard conditions (aqueous guanidine hydrochloride, pH 7.2, PhSH). Finally, removal of the substituted benzyl unit with acid gives the native peptide **21.6**, having Gly at the site of ligation (**21.4** → **21.6**).

Another approach to allow ligation at a non-cysteine residue, by means of an auxiliary, is based on the amines **22.1** (X = H or OMe) (Scheme 22).[47] The auxiliaries have the essential property of resisting cleavage under the acidic conditions used in peptide synthesis as long as they are attached to an *amine* nitrogen; however, after ligation and acyl transfer, the attachment is to an *amide* nitrogen (see **22.7**), and each auxiliary is now easily cleaved by acid.

The auxiliaries are attached to a resin-bound peptide (prepared by Boc-chemistry) via bromide displacement, as shown in Scheme 22 (**22.1** + **22.2** → **22.3**).[47] After deprotection and resin cleavage (**22.3** → **22.4**), reaction with a peptide (**22.5**) whose carboxyl terminus is in the form of a thioester results in ligation and acyl transfer (**22.4** → **22.6** → **22.7**). Finally, the auxiliary is removed, giving the native peptide **22.8**. When X = H, 95% HF and 5% *p*-cresol or CF_3CO_2H-Me_3SiBr are used to remove the auxiliary; when X = OMe the reagent system is 95% CF_3CO_2H, 2.5% Et_3SiH and 2.5% water.

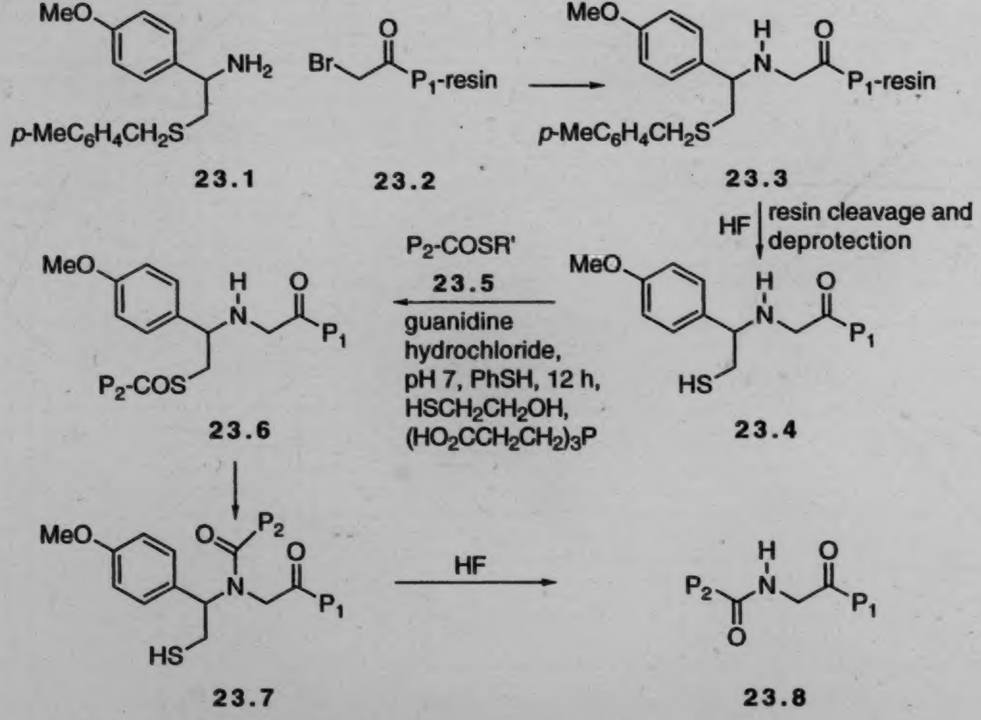

MeO
X
NH$_2$
p-MeC$_6$H$_4$CH$_2$S
22.1
Br
O
P$_1$-resin
22.2
H
N
22.3
acid cleavage and deprotection
P$_2$-COSR'
22.5
P$_2$-COS
P$_1$
22.6
HS
22.4
P$_2$
22.7
22.8

X = H or OMe, P$_1$ = peptide 1,
P$_2$ = peptide 2

Scheme 22

The approach was tested by the ligations of HN(Auxiliary)-Gly-Ser-Tyr-Arg-Phe-Leu-OH with thioesters of peptides containing 3, 31, 35 and 67 residues, the *C*-terminal thioester being Gly, Ala, Lys, and His, respectively. Ligation and acyl transfer involving Gly- or His-derived thioesters required 16 h, but with Ala- and Lys-derived thioesters 40 h were needed for comparable yields. In the case of the Ala-derived thioester, results were better when X = H than when X = OMe, but the difference was small (92% yield versus 85%).

The monomethoxy auxiliary has been used in the synthesis of cytochrome b562, which contains 106 amino acid residues


MeO
NH2
p-MeC6H4CH2S
23.1
Br
O
P1-resin
23.2
MeO
H
N
O
P1-resin
p-MeC6H4CH2S
23.3
HF
resin cleavage and deprotection
MeO
H
N
O
P1
HS
23.4
P2-COSR'
23.5
guanidine hydrochloride, pH 7, PhSH, 12 h, HSCH2CH2OH, (HO2CCH2CH2)3P
MeO
H
N
O
P1
P2-COS
23.6
MeO
O
P2
N
O
P1
HS
23.7
HF
H
N
O
P2
O
P1
23.8

P_2 = HN-A^{1}DLEDNXETLNDNLKVIEKADNAAQVKDALTKMRAAALDAQKATPPKL-EDKSPDSPEMKDFRH^{63}, X = Met or SeMet

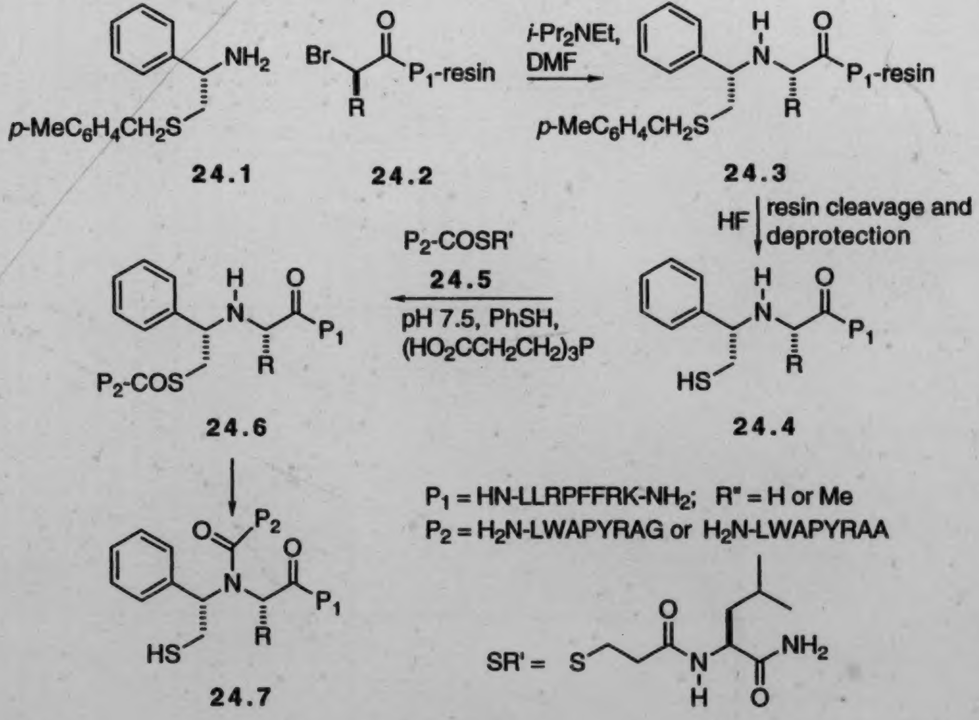
P_1 = HN-F^{65}DILVGQIDDALKLANEGKVKEAQAAAEQLKTTRNAYHQKYR^{106}-OH

Scheme 23

and has no Cys (Scheme 23).[42]

The thioester segment **23.5** was made by solid phase peptide synthesis using Boc-chemistry on a thioester-generating resin, and the other segment (**23.2**) was also made on a resin. Nucleophilic displacement then served to attach the auxiliary (**23.2** → **23.3**). Following deprotection and cleavage from the resin the two segments were used for ligation and acyl transfer, and the auxiliary was removed with HF. The corresponding [SeMet^7]cytb562 was also made.

The use of benzylamine auxiliaries introduces a new asymmetric center on the modified *N*-terminus, and the effectiveness of both isomers has been examined.[49] The auxiliary **24.1** and its enantiomer were each incorporated as the *N*-terminus of a polypeptide on a solid support by bromide displacement with inversion to produce, in the case of enantiomer **24.1**, the *N*-protected peptide **24.3**. Deprotection and cleavage from the resin gave **24.4**, which was subjected to transthioesterification with **24.5** under standard conditions for acyl transfer. The residual auxiliary on the product (**24.7**) is not removable. Two auxiliary-modified *N*-terminal amino acids were studied – Gly (R = H in **24.3**) and Ala (R = Me in **24.3**), and for each one both enantiomers of the



NH2
p-MeC6H4CH2S
24.1
O
Br
P1-resin
R
24.2
i-Pr2NEt, DMF
H
N
O
P1-resin
R
p-MeC6H4CH2S
24.3
HF
resin cleavage and deprotection
P2-COSR'
24.5
pH 7.5, PhSH, (HO2CCH2CH2)3P
H
N
O
P1
R
P2-COS
24.6
H
N
O
P1
R
HS
24.4
O
P2
N
O
P1
R
HS
24.7
P1 = HN-LLRPFFRK-NH2; R" = H or Me
P2 = H2N-LWAPYRAG or H2N-LWAPYRAA
SR' = S
O
N
H
NH2
O

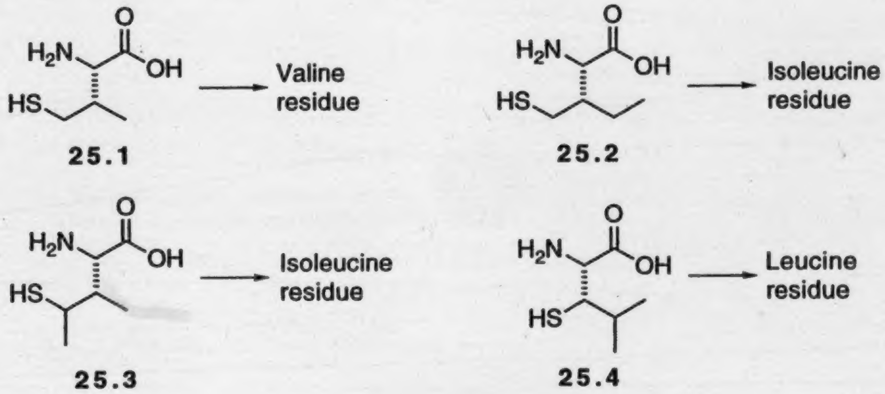
Scheme 24

auxiliary were tested. For a derivatized Gly terminus the ligation and acyl transfer rate is independent of the stereochemistry of the auxiliary, at least for reaction with a Gly thioester or Ala thioester. However, when the auxiliary (either enantiomer) was attached to an Ala residue (R = Me in **24.4**), only the initial ligation occurred with a Gly thioester and an Ala thioester, but not the acyl transfer. The effect of pH and solvent was not investigated, however.

Other studies have shown[18] that as far as the thioester component is concerned, only the β-branched amino acids and Pro have ligation rates significantly slower than Ala. In the reactions of Scheme 24 the acyl transfer is rate-determining, and addition of PhSH, commonly added to activate thioesters for ligation, had no effect.

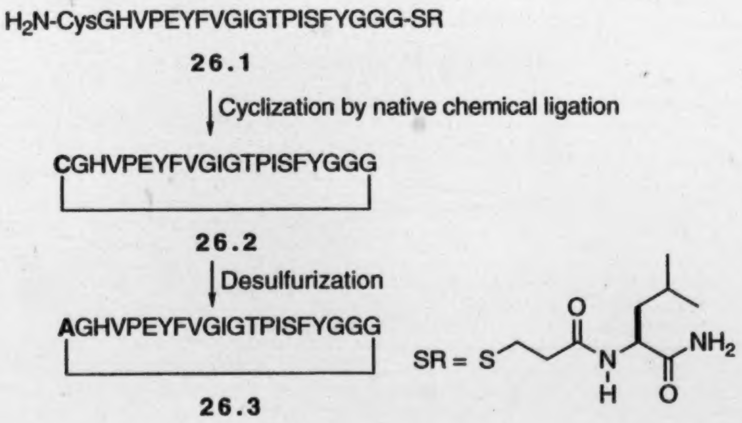
(c) Conversion of Cys at the ligation site into Ala[50]

The utility of the standard native chemical ligation has been extended by the conceptually simple method of desulfurizing the Cys after ligation, so as to generate an Ala residue. It should be noted that Ala is one of the most abundant amino acid residues in proteins.[50] The desulfurization was best effected with Raney nickel. Of course, other Cys residues (even Acm-protected Cys) cannot be present in the target peptide (they would also be desulfurized), and care must be exercised to control the reaction time when Met is present, as this residue is itself slowly desulfurized. Unnatural sulfhydryl amino acids, such as **25.1-25.4**, can be used, since these afford natural amino acids at the site of ligation on desulfurization. This possibility was demonstrated by coupling AcHN-M^{1}TYKLILNGKTLKGETTTEAVDA^{23}-SR with HomoCys-AYGGFL-NH$_2$; desulfurization of the product gave AcHN-M^{1}TYKLILNGKTLKGET-TTEAVDA-Abu-AYGGFL-NH$_2$ (Abu = α-aminobutyric acid residue). As indicated in Scheme 25, unnatural amino acids **25.1-25.4** would lead to Val, Leu, or Ile residues at the ligation site instead of Cys.


H2N
O
OH
HS
Valine residue
25.1
H2N
O
OH
HS
Isoleucine residue
25.2
H2N
O
OH
HS
Isoleucine residue
25.3
H2N
O
OH
HS
Leucine residue
25.4

Scheme 25

The method was first demonstrated by synthesis of microcin J25 (**26.3**), a small cyclic peptide containing 21 amino acid residues. It contains one Ala but no Cys. A linear analog of microcin J25 was made that had a *C*-terminal thioester and an *N*-terminal Cys in place of the natural Ala. Native chemical ligation gave a cyclic analog and desulfurization afforded the natural material (Scheme 26).


H2N-CysGHVPEYFVGIGTPISFYGGG-SR
26.1
Cyclization by native chemical ligation
CGHVPEYFVGIGTPISFYGGG
26.2
Desulfurization
AGHVPEYFVGIGTPISFYGGG
26.3
SR = S
O
N
H
NH2
O

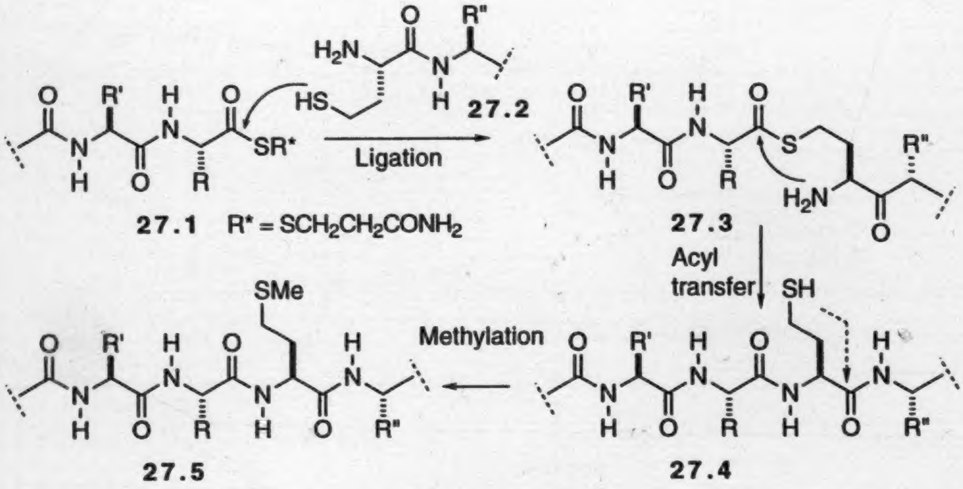
Scheme 26

Peptide **26.1** was made by solid phase methods. When exposed to the standard conditions of native chemical ligation the cyclized peptide **26.2** was formed (50% isolated yield). Treatment with Pd/Al_2O_3 in 20% aqueous AcOH served to convert the Cys into an Ala, giving the natural peptide **26.3** (52%).

The above method was applied to a linear peptide called PGB1. This is a 56-amino acid immunoglobulin binding domain from *Streptococcus.* It contains six Ala residues but no Cys.[51] The *N*-terminal thioester segment used corresponds to residues 1-23 [AcHN-M^1TYKLILNGKTLKGETTTEAVDA^{23}-SR] and the *C*-terminal segment corresponds to residues 24-56, in which Ala^{24} was replaced by Cys in order to permit ligation. The ligated product N^{α}-Ac[Cys^{24}]PGB1 was subjected to desulfurization with Pd/Al_2O_3 in a buffer, affording N^{α}-Ac-PGB1 in 80% yield. The desulfurization was selective, and the methionine of N^{α}-Ac-PGB1 did not react. Similarly, [Cys^{49}]barnase,[4] an analog of the natural peptide, was converted into [Ala^{49}]barnase by desulfurization with Pd/Al_2O_3. These experiments showed that desulfurization is compatible with all the natural amino acids.

(d) Conversion of homocysteine at the ligation site into Met

The above desulfurization method is related to earlier work[52] in which ligation was effected using homocysteine (Hcy) instead of Cys, and the resulting thiol at the ligation site was methylated so that a Met was ultimately present at the ligation site (Scheme 27).



Scheme 27

Typically, the ligation is done at pH 7.6 in the presence of an excess of $(HO_2CCH_2CH_2)_3P$, which prevents disulfide formation and which also accelerates the reaction. Transthioesterification and acyl migration were usually complete within 4 h. A number of model peptides were made from the thioesters and *N*-terminal Hcy peptides, as shown in Scheme 28. The peptides were made by solid phase methods, using Boc-chemistry for the thioesters (thioesters are attacked by the nucleophiles used repetitively to remove Fmoc groups) and either Boc- or Fmoc-chemistry for the *N*-terminal Hcy peptides.

Thioesters	***N*-Terminal Homocysteinyl peptides**
H_2N-KLYG-SR	H_2N-Hcy-KLQDV-OH
H_2N-KLYG-SR	H_2N-Hcy-ARVELKKLQDV-OH
H_2N-KLYG-SR	H_2N-Hcy-ERVEWLRKKLQDVHNF-OH
H_2N-KYGGFL-SR	H_2N-Hcy-KLQDV-OH
H_2N-KYGGFL-SR	H_2N-Hcy-ARVELKKLQDV-OH
H_2N-KYGGFL-SR	H_2N-Hcy-ERVEWLRKKLQDVHNF-OH
H_2N-SVSEIQLMHNLGKHLNS-SR	H_2N-Hcy-KLQDV-OH
H_2N-SVSEIQLMHNLGKHLNS-SR	H_2N-Hcy-ARVELKKLQDV-OH
H_2N-SVSEIQLMHNLGKHLNS-SR	H_2N-Hcy-ERVEWLRKKLQDVHNF-OH

$R = SCH_2CH_2CONH_2$

Scheme 28

In addition, two *N*-terminal Hcy peptide thioesters, H_2N-Hcy-KYGGFL-SR and H_2N-Hcy-SVSEIQLMHNLGGKHLNS-SR, were subjected to the ligation conditions and were found to undergo cyclization.

Several side reactions were observed, but can be minimized by proper attention to the experimental procedure. It should be noted that the initial products are susceptible to degradation caused by attack of the Hcy sulfhydryl group on the adjacent carbonyl via a five-membered transition state (see **27.4**, dotted arrow).

The products from these ligations were methylated on sulfur, using methyl *p*-nitrobenzenesulfonate, reaction being stopped before methylation of lysine ε-amino groups or of imidazole rings occurred.

(e) Conversion of Cys at the ligation site (and elsewhere) into dehydroalanine[53]

A number of peptides – mainly cyclic – containing from 5-14 amino acids were made by native chemical ligation (Scheme 29) and all the Cys residues were then converted into dehydroalanine residues. The conversion was effected by *S*-cyanation (-SH → -S-CN) with 1-cyano-4-dimethylaminopyridinium tetrafluoroborate, followed by elimination by treatment with *i*-Pr_2NEt. Alternatively, each thiol group was methylated and then oxidized to a sulfoxide, which underwent elimination on treatment with DBU. The fact that Cys not at the ligation site is also changed is a limitation of this method.

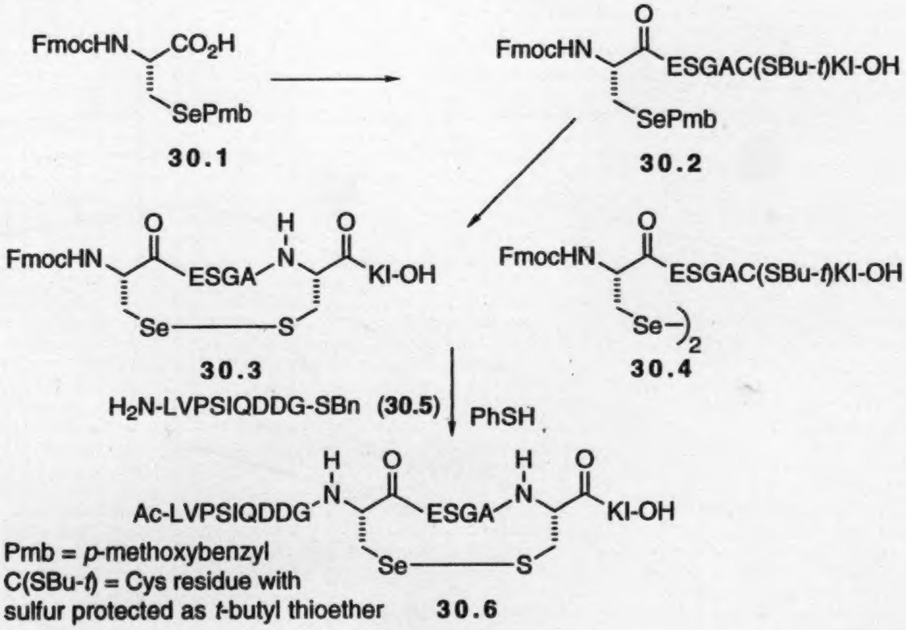
	Thioester	**Product**
1	H_2N-CAGFY-SR	c[CAGFY]
2	H_2N-CSLKLNG-SR	c[CSLKLNG]
3	H_2N-CKYSSRGISWSYL-SR	c[CKYSSRGISWSYL]
4	H_2N-CKYSSRGICWSYL-SR	c[CKYSSRGICWSYL]
5	H_2N-SLKLNG-SR + H_2N-CNSFRY-OH	H_2N-SLKLNGCNSFRY-OH

-SR = $-SCH_2CH_2CONH_2$

Scheme 29

(f) Selenocysteine at the ligation site

Several studies have been reported on the use of selenocysteine instead of Cys. The protected selenocysteine **30.1** has been incorporated as the *N*-terminus of a short peptide (see **30.2**).[54] Oxidative deprotection of the selenium, using iodine, gave a mixture of **30.3** and **30.4**, and both of these underwent ligation and acyl transfer when treated with the thioester **30.5** in the presence of PhSH, which generates the free selenol from either **30.3** or **30.4**. The reactions took 24 h under the conditions used; and, given the greater acidity of a selenol compared with a thiol, the more nucleophilic nature of a selenolate, and the fact that aminolysis of selenoesters is much faster than of thioesters,[55] it is surprising that the ligations were not faster. Possibly, the rate-limiting step is formation of the

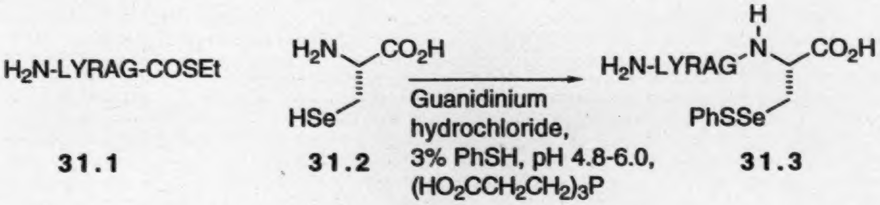

FmocHN
CO2H
SePmb
30.1
FmocHN
ESGAC(SBu-t)KI-OH
SePmb
30.2
FmocHN
ESGA
KI-OH
Se
S
30.3
FmocHN
ESGAC(SBu-t)KI-OH
Se
2
30.4
H2N-LVPSIQDDG-SBn (30.5)
PhSH
Ac-LVPSIQDDG
ESGA
KI-OH
Se
S
30.6

Pmb = *p*-methoxybenzyl
C(SBu-*t*) = Cys residue with
sulfur protected as *t*-butyl thioether

Scheme 30

free selenol from **30.3** or **30.4**. The use of selenocysteine might offer some opportunities for further modification of the final products by deselenation (to generate an Ala residue), but this does not appear to have been examined.

A more advanced study of selenocysteine-mediated native chemical ligation has also been reported.[55]

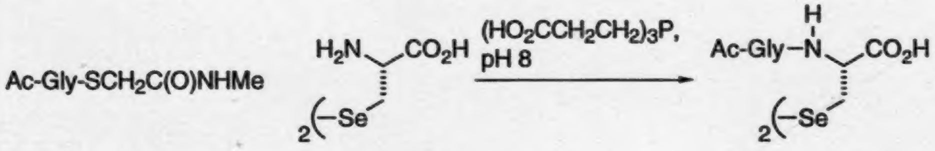

H2N-LYRAG-COSEt
31.1
H2N
CO2H
HSe
31.2
Guanidinium hydrochloride, 3% PhSH, pH 4.8-6.0, (HO2CCH2CH2)3P
H
N
H2N-LYRAG
CO2H
PhSSe
31.3

Scheme 31

The pentapeptide thioester **31.1** underwent smooth ligation and acyl transfer with selenocysteine (**31.2**), generated *in situ* from selenocystine by reaction with $(HO_2CCH_2CH_2)_3P$, giving **31.3** (Scheme 31).[55] The corresponding reaction with Cys went at about the same speed. Selenocysteine-mediated native chemical ligation was then used[55] to make a selenocysteinyl derivative of bovine pancreatic trypsin inhibitor, a 58-amino acid polypeptide that is an inhibitor of serine proteases whose natural form had previously been made by standard native chemical ligation.[25] Conventional[56] Fmoc solid-phase peptide synthesis on a Pam resin, followed by cleavage from the resin by treatment with Me_3Al and EtSH, gave the required 37-amino acid thioester segment H_2N-R^1PDFCLEPPYTGPCKARIIRYFYNAKAG-LCQTFVYGG^{37}-COSEt. The other segment (H_2N-U^{38}RAKRNNFKSAEDCM-

RTCGGA^{58}-OH; U = selenocysteine) was synthesized on a Wang resin, using *p*-methoxybenzyl protection for the selenium during assembly of the peptide. The material was obtained as a mixed selenosulfide formed between Sec^{58} (Sec = selenocysteine) and either Cys^{51} or Cys^{55}. The ligation was effected under similar conditions to those used for the model study summarized in Scheme 31.

Another advanced study on the use of Sec^{57} in place of Cys led to the synthesis of a peptide with 124 amino acid residues.



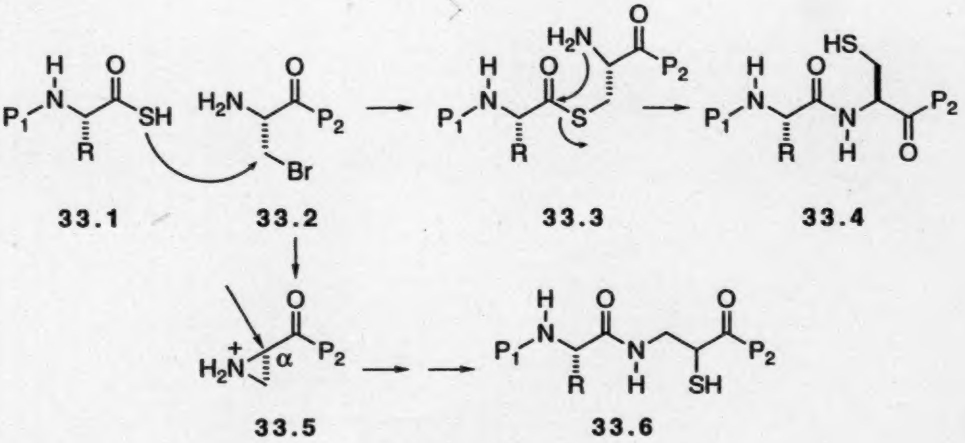
32.1 **32.2** **32.3**

Scheme 32

First, the simple model ligation shown in Scheme 32 was performed, and worked without incident. In a related model, the ligation was 10^3 faster with Sec than with Cys at pH 5, as expected from the greater nucleophilicity of RSe^- compared with RS^-, the lower pK_a of RSeH than RSH, and faster aminolysis of selenoesters than thioesters. Next, a selenium analog of ribonuclease A was made. A fragment corresponding to residues 1-109 with a *C*-terminal thioester was made by recombinant DNA technology, and standard solid phase methods were used to synthesize a peptide corresponding to residues 110-124, but with Sec at position 110. The two segments were ligated, and the resulting protein was folded to material that had the same activity as wild-type ribonuclease A.

Variations of the native chemical ligation that still afford a native peptide backbone

(a) use of an N-terminal β-bromoalanine

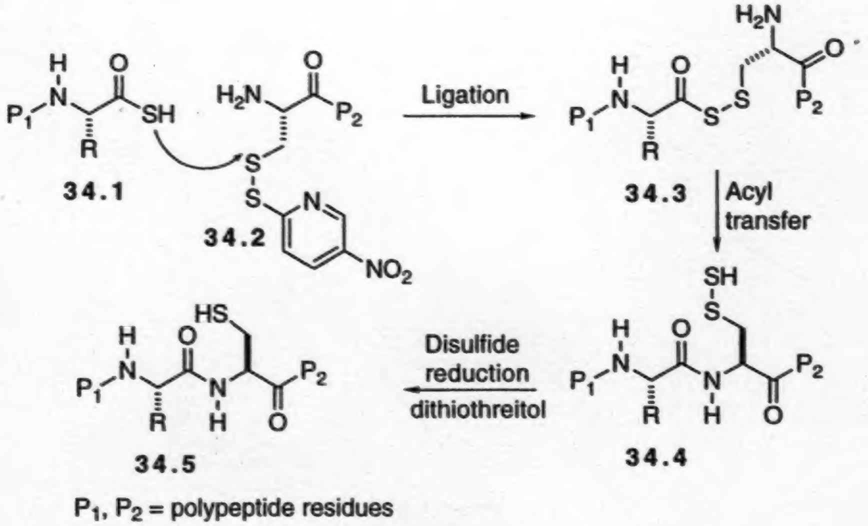


Scheme 33

Another method that accomplishes the same overall result as linking an *N*-terminal Cys with a *C*-terminal thioester is summarized in Scheme 33.[23] Here a thioacid (**33.1**) is used to displace bromine from an *N*-terminal β-bromoalanine (**33.2**). The resulting intermediate, which is identical to that obtained by the thioester-Cys approach, rearranges to the native peptide **33.4**. Some of the undesired ligation-rearrangement product **33.6** was also formed by initial attack on the aziridinium ion **33.5** at C^{α}. This pathway was suppressed at low pH (3% at pH 4.7, 40% at pH 6.2) in the case of coupling the single amino acids Leu-SH and BrAla. The β-bromoalanine method was used to couple SAKL$^{\alpha}$COSH with BrAlaPGGNAC(Acm)V-OH, the coupled product being obtained in 85% yield.

(b) *Use of acyl disulfides*

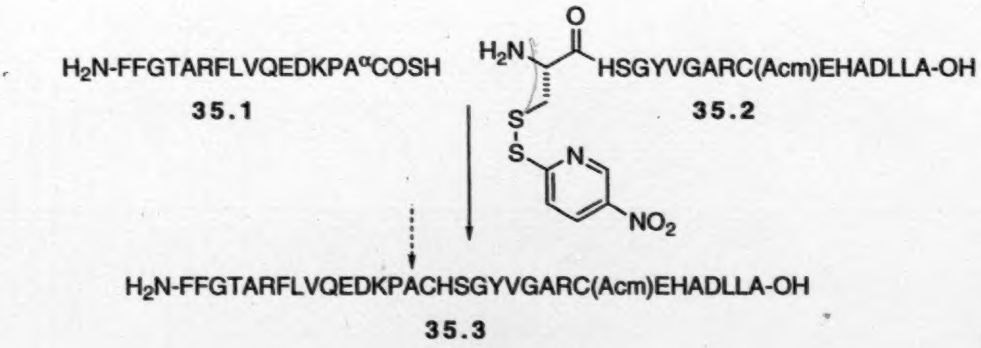
An alternative way of ligating two peptide segments is summarized in Scheme 34.[58]


Ligation
34.1
34.2
34.3
Acyl transfer
34.4
Disulfide reduction
dithiothreitol
34.5
P_1, P_2 = polypeptide residues

Scheme 34

In this approach the $^{\alpha}$COSH reacts with a derivatized *N*-terminal Cys to form an acyl disulfide **34.3**. This undergoes intramolecular rearrangement via a six-membered transition state (**34.3** → **34.4**). Finally, reduction of the disulfide link in **34.4**, using dithiothreitol, releases the native peptide with a Cys at the ligation site. The method was tested by synthesis of a 32-residue model peptide (Scheme

35).

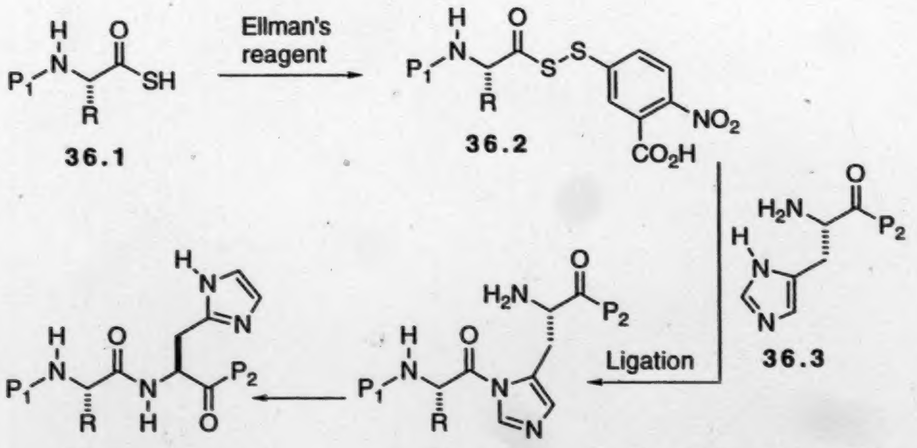

H2N-FFGTARFLVQEDKPAαCOSH
35.1
H2N
O
HSGYVGARC(Acm)EHADLLA-OH
35.2
S
S
N
NO2
H2N-FFGTARFLVQEDKPACHSGYVGARC(Acm)EHADLLA-OH
35.3

Scheme 35

The individual peptides were made by solid phase methods and the thiopyridyl unit was attached to the *N*-terminal Cys by reaction with 2,2′-dithiobis(5-nitropyridine). When solutions of **35.1** and **35.2** were mixed in aqueous MeCN at pH 2, the initial ligation occurred immediately. The pH was then adjusted to pH 6 and, after a further 10 min reduction with dithiothreitol gave the final product **35.3**. The whole process is reported to be efficient, but no yield was given. The site of ligation in **35.3** is marked by an arrow.

Acyl disulfides related to **34.2**, but lacking the nitro substituent have also been used [59] Two peptide thioacids H_2N-YSAELV-SH and H_2N-YSAELG-SH were coupled with H_2N-C($^{\beta}$S-pyridyl)YSELA-NH_2 in good yield (>75%) within 20 min.[59]

A different method of using acyl disulfides was also examined,[60] but in this case the acyl terminus was activated and the other segment carried a His residue at its *N*-terminus instead of Cys (Scheme 36).



P_1, P_2 = polypeptide residues

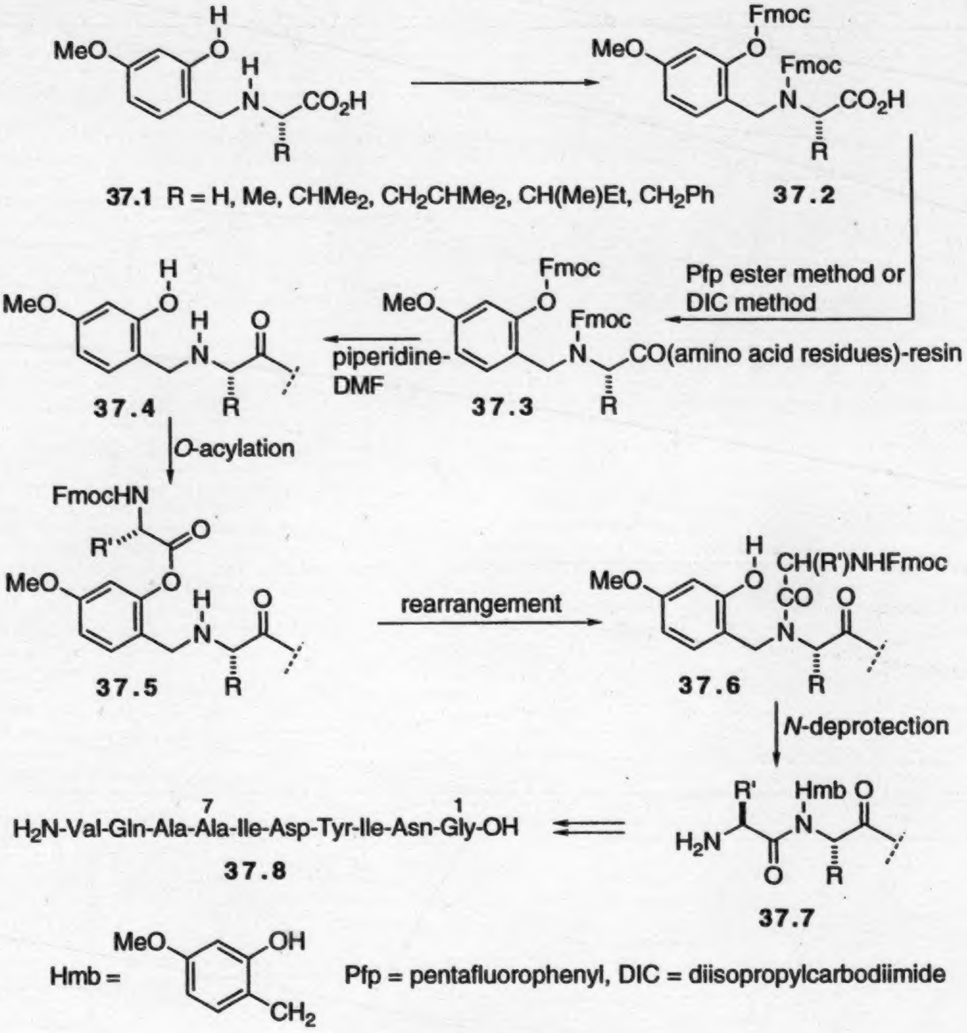
$H_2N\text{-SKAL}^{\alpha}\text{COSH}$ (**36.6**) $H_2N\text{-HGKA-OH}$ (**36.7**) $\longrightarrow$ $H_2N\text{-SKALHGKA-OH}$ (**36.8**)

Scheme 36

Both segments **36.1** and **36.3** were made by standard solid phase synthesis. No significant coupling took place when the segments were mixed together, but addition of Ellman's reagent [5,5'-dithiobis(2-nitrobenzoic acid)] led to the coupled native peptide. The mechanism shown in Scheme 36 has been suggested, but there is disagreement[61] about the nature of the species formed from the thioacid (**36.1**) and Ellman's reagent. The scheme was tested with simple models: coupling of **36.6** and **36.7** was performed at pH 5.7 in 1:1 water-DMF and gave the expected product **36.8** in 75% yield. In the simple model studies, **36.2** reacted selectively with *N*$^{\alpha}$-amines rather than with the *N*$^{\varepsilon}$-group of lysine. A 25-residue peptide was made by this method, this time in 60% yield, by coupling **36.6** with H_2N-HLSSMERVEWLRKKLQDVHNF-OH. It should be noted that an internal His is compatible with this methodology, as demonstrated by the last example.

(c) Use of amide nitrogen backbone protection

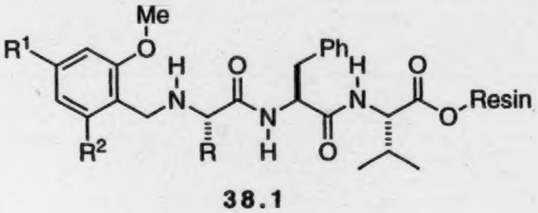
Studies aimed at facilitating the synthesis of so-called[62] "difficult sequences" by solid phase peptide synthesis have examined the effect of preventing hydrogen bonding of N-H bonds by alkylating the amide nitrogen.[63] Introduction of tertiary amide bonds in peptides is most logically achieved by using precursor amino acids in which the nitrogen carries a removable alkyl group. However, for amino acids other than glycine, this type of substitution usually causes serious steric hindrance to subsequent peptide bond formation.[63] Sheppard and co-workers[63,64,65] have developed an *N*-modifying group (Scheme 37) in which the effects of steric hindrance are offset by a mechanism of acyl capture and *O* → *N* acyl transfer (**37.4** → **37.5** → **37.6**). Thus, the *N*-alkyl-substituted amino acid **37.2**[63,66] was coupled with the *N*-terminus of a resin-bound peptide so as to form **37.3**. The coupling was done with diisopropylcarbodiimide or by use of the pentafluorophenyl ester of **37.2**. Removal of the Fmoc protecting groups and *O*-acylation gave the intermediate **37.5**, which underwent *O* → *N* acyl transfer to **37.6**. Removal of the Fmoc group and acylation of the *N*-terminus in the usual way, followed by removal of the 2-hydroxy-4-methoxybenzyl group (with CF_3CO_2H[66]) and resin cleavage then releases the peptide. The value of the


37.1 R = H, Me, CHMe2, CH2CHMe2, CH(Me)Et, CH2Ph
37.2
Pfp ester method or DIC method
CO(amino acid residues)-resin
piperidine-DMF
37.3
37.4
O-acylation
37.5
rearrangement
37.6
N-deprotection
37.7
H2N-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-OH
37.8
Hmb =
Pfp = pentafluorophenyl, DIC = diisopropylcarbodiimide

Scheme 37

approach summarized in Scheme 37 was shown by synthesis of the well-known "difficult sequence" acyl carrier protein 65-74 decapeptide **37.8**. This sequence undergoes strong inter-chain association after addition of the penultimate Gln residue; addition of the final Val is strongly hindered and is invariably 10-15% incomplete under standard conditions (pentafluorophenyl ester-HOBT couplings, 45 min in DMF).

Insertion of the *N*-substituted Ala derivative **37.1** (R = Me) at residue 7, enabled the final Val (residue 10) to be coupled completely under the standard conditions. The long-range effect of *N*-substitution confers flexibility in the choice of residue to be replaced. However, since *O*-acylation is intrinsically slower than *N*-acylation, the sequence of *O*-acylation followed by *O* → *N* migration will inevitably be slower than direct *unhindered* *N*-acylation, and it would be unwise to choose unnecessarily an intrinsically hindered site containing β-branched residues. The possible steric constraints were examined by studying the acylation of **37.4** (R = H, Me, $CHMe_2$, CH_2CHMe_2, CH(Me)Et, CH_2Ph) with a range of Fmoc-amino acid anhydrides. When R = H in **37.4**, acylation was fast (complete within 1 h) with a variety of residues including Val, Ile, and *O-tert*-butylthreonine, and so any of the common amino acids (as symmetrical anhydrides) can be used.[63,64] In the case of **37.4** [R = CH(Me)Et] 20 h were required for complete acylation with unhindered residues (Gly, Ala, *tert*-butyl glutamate), and acylation with Leu, N^{ε}-Boc-lysine, and *O-tert*-butyl serine was still incomplete after 20 h. *O-tert*-butyl threonine (as its symmetrical anhydride) failed to react appreciably; compound **37.4** (R = $CHMe_2$) behaved similarly. These observations indicate that Val, Ile, and probably *O-tert*-butylthreonine should not be chosen for *N*-substitution with the auxiliary unless the next residue to be coupled is unhindered. Residues with substituents of intermediate size (**37.4**, CH_2CHMe_2) may require extended reaction times for addition of amino acids other than Gly, and so should not be chosen if the following residue has a β-branched structure. Pentafluorophenyl esters can also be used for coupling to the derivatized *N*-terminus.[64]



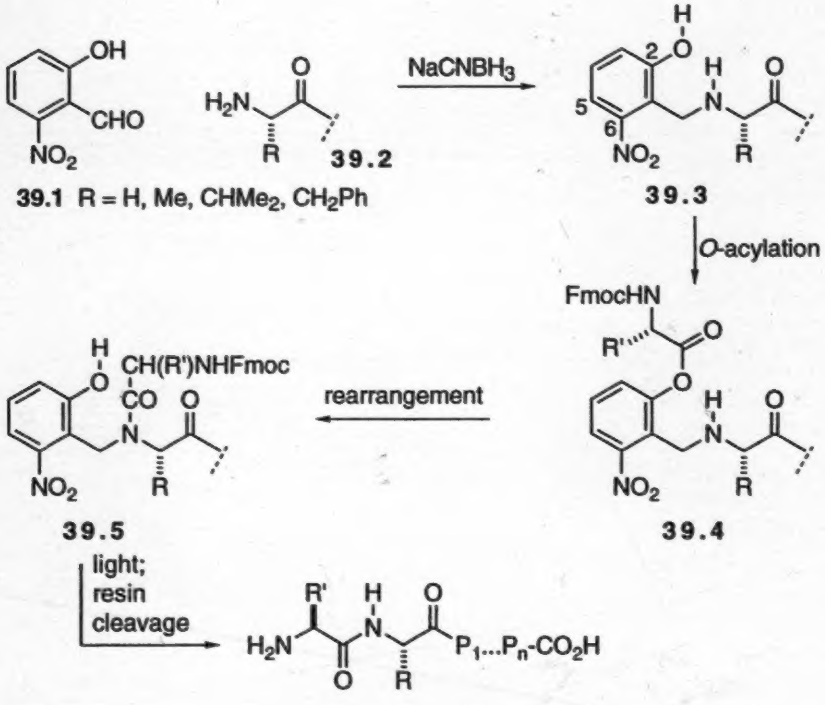
38.1

Scheme 38

In the above work, several observations were made about the effect of substituents R^1 and R^2 in compounds of type **38.1** (see Scheme 38). The trimethoxy species **38.1** ($R^1 = R^2 = OMe$) was more rapidly acylated than the less hindered **38.1** ($R^1 = OMe$, $R^2 = H$). Possibly, *ortho* methoxy substitution produces steric factors that result in a conformation that is more favorable for acylation or ensures that there is intramolecular hydrogen bonding with the adjacent N-H. However, how these possibilities enhance the rate of acylation is not clear.

The methoxy-substituted auxiliary (see **37.1**) is too acid-labile[67] for use in Boc- and benzyl-based methods but, in the *absence* of the methoxy group, the backbone protection is suitable for Boc- and benzyl protocols because the acid stability of the protecting group is now greater.[67] The desmethoxy protecting group can be removed with CF_3SO_2H, but the coupling rate is lower.[67]

With the 2-hydroxy-4-methoxybenzyl auxiliary (see **37.1**), the *O* → *N* acyl transfer can be slow when hindered residues are involved[68, 69] and, as implied above there is a need for a new auxiliary with superior acyl transfer efficiency if the full potential of amide-backbone substitution is to be realized. An improved version has been developed by judicious placement of a nitro group on the benzene ring (Scheme 39).

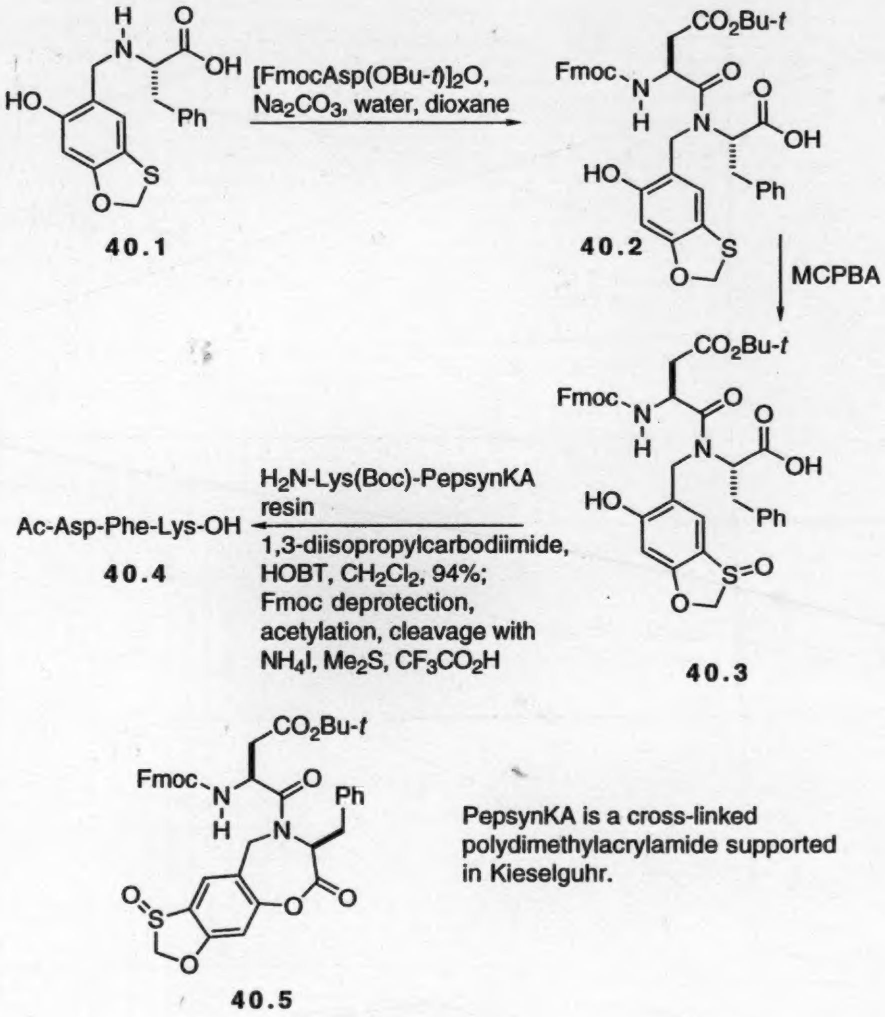

OH
CHO
NO2
H2N
O
R
39.2
39.1 R = H, Me, CHMe2, CH2Ph
NaCNBH3
H
O
2
5
6
N
39.3
O-acylation
FmocHN
R'
39.4
rearrangement
CH(R')NHFmoc
CO
39.5
light; resin cleavage
R'
P1...Pn-CO2H
39.6

Scheme 39

The function of the auxiliary is threefold: *O*-acylation is first required, and then *O* → *N* acyl transfer must occur, and both steps must be efficient, irrespective of the nature of the amino acids involved. Finally, the auxiliary must be removable. The 6-nitro-2-hydroxybenzyl group (Hnb; see **39.3**) was found to be the best of several that were examined; the corresponding 5-nitro analog satisfied the first two requirements, but not the last. The 6-nitro-2-hydroxybenzyl group is attached to the *N*-terminus of a resin-bound peptide by reductive alkylation (**39.1** → **39.3**). *O*-acylation with amino acids, using the HBTU-mediated method,[70] proceeds rapidly (less than 1 min for HBTU-activated Ala and Phe, and 10 min for more than 95% acylation with the Val HBTU derivative). Subsequent *O* → *N* acyl transfer is also rapid. The Hnb group is removed by photolysis at 366 nm in MeOH in the presence of amine scavengers, such as Lys.

The effectiveness of the Hnb auxiliary in facilitating the assembly of "difficult" sequences was demonstrated by comparing the stepwise assembly of TGYIKTELISV, using standard Fmoc and *tert*-butyl solid phase methods — a process that gave an unacceptable average acylation yield of 83% — with assembly involving incorporation of the Hnb group on the third residue (Ile) from the resin linker. The average acylation yield for the preparation of TGYIKTELI(Hnb)SV was then 99.6%. Photochemical removal of the auxiliary was accomplished in 76% yield. Use of the 2-hydroxy-4-methoxybenzyl (Hmb) auxiliary was much less successful, as coupling of Leu to the Hmb-derivitized Ile terminus (during synthesis of TGYIKTELISV) was only 21% complete in 24 h, while the corresponding coupling using the Hnb auxiliary was quantitative. The Hnb auxiliary has also been used to facilitate cyclization of linear peptides.[71]

As described above, the method developed by Sheppard and co-workers, applies to the sequential addition of single amino acids, but backbone protection has also been used for assembly of protected peptide segments.[5] However, when the 2-hydroxy-4-methoxybenzyl group is used to protect the *C*-terminal amide bond of a fully protected segment, the rate of coupling of that *C*-terminus to the *N*-terminus of the other segment is low. This is due to formation of a 4,5-dihydro-8-methoxy-1,4-benzoxazepin-2(3*H*)-one species (cf. **40.5**) between the activated carboxyl group and the phenolic hydroxyl of the *N*-substituent; the benzoxazepinone is not a powerful acylating agent. In order to overcome this problem, an electron-withdrawing group has been placed *para* to the phenolic hydroxyl. The approach was tested in the following way. The *N*-alkylated amino acid **40.1** was coupled in the usual way with the anhydride made from FmocAsp(OBu-*t*)OH, and the product (**40.2**) was oxidized on sulfur (**40.2** → **40.3**). Coupling of this dipeptide (94%) with a resin-supported (PepsynKA is a cross-linked polyacrylamide supported on Kieselguhr[63]) Lys, followed by deprotection and cleavage, gave **40.4**. The deprotection sequence involved reduction of the (electron-withdrawing) sulfoxide to a sulfide, in order to confer acid lability. Very little (<0.25%) epimerization was observed (at the Phe site) in the coupling of the peptide

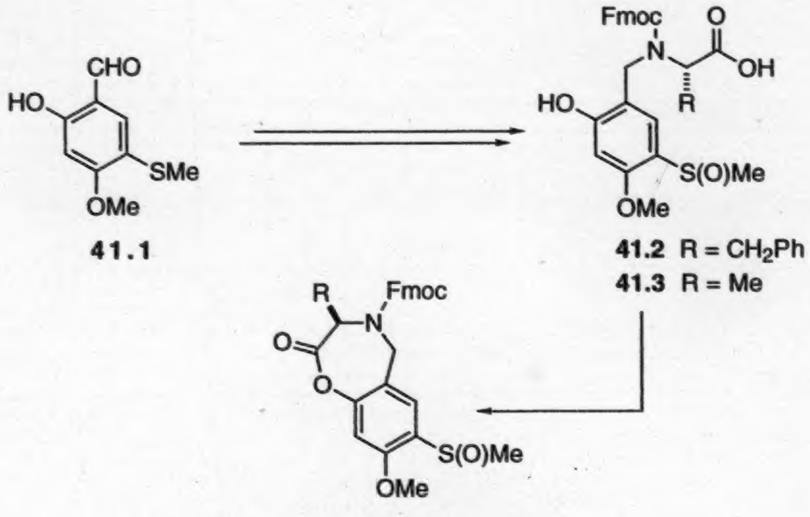

[FmocAsp(OBu-*t*)]$_2$O, Na$_2$CO$_3$, water, dioxane
MCPBA
H$_2$N-Lys(Boc)-PepsynKA resin
1,3-diisopropylcarbodiimide, HOBT, CH$_2$Cl$_2$, 94%; Fmoc deprotection, acetylation, cleavage with NH$_4$I, Me$_2$S, CF$_3$CO$_2$H
Ac-Asp-Phe-Lys-OH
40.1
40.2
40.3
40.4
40.5

PepsynKA is a cross-linked polydimethylacrylamide supported in Kieselguhr.

Scheme 40

segment **40.3** with the Lys.[5] The experiment serves as a model for coupling of peptide segments without the traditional danger of epimerization at the *C*-terminus.

More recently, a related auxiliary (Scheme 41) was reported in which the sulfoxide is not part of a ring.[72] In this modification the derivatized amino acids **41.2** and **41.3** are easily prepared (by a process involving reductive amination of aldehyde **41.1**), and they couple quantitatively through standard uronium activation, via an intermediate benzoxapin-2(3*H*)-one (**41.4**). Intermediate **41.4** is a better acylating agent than the corresponding species with H instead of S(O)Me.



41.1

41.2 R = CH_2Ph
41.3 R = Me

41.4

41.5 $\mathrm{H_2N}$-Tyr-Phe-$\overset{*}{\text{Phe}}$-Gly-Leu-OH

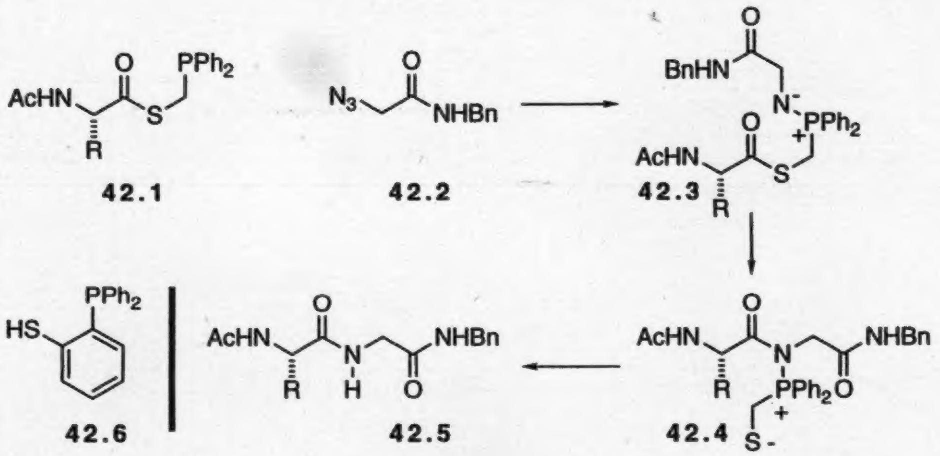
41.6 $\mathrm{H_2N}$-Val-Gln-Ala-$\overset{*}{\text{Ala}}^{68}$-Ile^{69}-Asp-Tyr-Ile-Asn-Gly-OH

Scheme 41

The system was tested by use of **41.2** in the solid phase stepwise synthesis of **41.5**, and **41.3** was used in the synthesis of **41.6** (the derivatized amino acids are indicated by asterisks). In the former case, coupling of Phe onto the derivatized terminus was achieved using pentafluorophenyl ester chemistry and a reaction time of 45 min. Comparable coupling of Phe to **37.4** (see Scheme 37, R = Bn) required 24 h with symmetrical anhydrides. The synthesis of **41.6** illustrated the application to a well-known difficult sequence (residues 65-74) from acyl carrier protein. Here, **41.3** was added after Ile^{69} via BOP/HOBt/NMM activation (45 min). Removal of the Fmoc group was followed by *N*-acylation using Fmoc-Ala-OC_6F_5/HOBt in a standard 45-min coupling. Continuation of the sequence gave the desired decapepetide, with no detectable level of the des-Val nonapeptide.

(d) Use of Staudinger ligation

The thioesters **42.1** (R = H, Ph) reacted in aqueous THF at room temperature with azide **42.2** by the normal mechanism of the Staudinger reaction to give, presumably, the ylide **42.3**.[73] This underwent spontaneous rearrangement through a five-membered transition state to **42.4**, and hydrolysis then released the peptide **42.5** (80% for R =H, 92% for R = Ph). The efficiency of the reaction, using thioesters derived from



Scheme 42

$HSCH_2PPh_2$ was much higher than with those derived from **42.6**; in the later case the acyl transfer is by way of a six-membered ring. Thioesters **42.1** also have an intrinsic advantage over those derived from **42.6**[74]; aliphatic thioesters are more resistant to hydrolysis in aqueous solution, and such hydrolysis is likely to be a competing side reaction. The Staudinger ligation has also been examined with azides derived from Phe, Asp, and Ser without detectable epimerization.[75] These amino acids have a moderate (Phe) to high (Asp, Ser) propensity for epimerization in standard peptide couplings.[75]

The above type of ligation has yet to be tested in synthesis of large peptides.

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62 Reference 3d, especially p 969.

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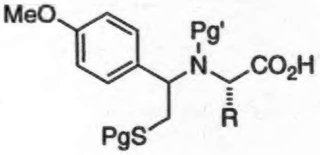
DISCUSSION OF RESEARCH RESULTS

Studies on the design of a general auxiliary

Our aim was to prepare compounds of type **1.1** for each of the common amino acids, as such derivatives might be useful in a general ligation and acyl transfer approach to the synthesis of proteins. If an amino acid residue derived from **1.1** could be installed as the *N*-terminus of a peptide segment then removal of the nitrogen and sulfur protecting groups and reaction with another peptide segment having a *C*-terminal thioester should result in thioester exchange, followed by *S* → *N* acyl transfer. Removal of the auxiliary would then give the native peptide. We hoped that ligation would be possible by this scheme for any value of R in **1.1**.



1.1

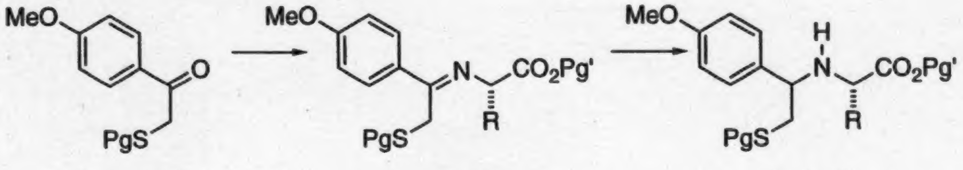
Scheme 1

A number of approaches to **1.1** were examined and, eventually, a synthetic route was developed and applied to three amino acids. Many exploratory experiments were carried out before a successful route was found; these preliminary experiments are not described in the Experimental Section, and often full characterization data were not obtained. However, the route that was ultimately successful is reported in detail in the Experimental Section.

Approaches to compounds of type 1.1

Imine approach

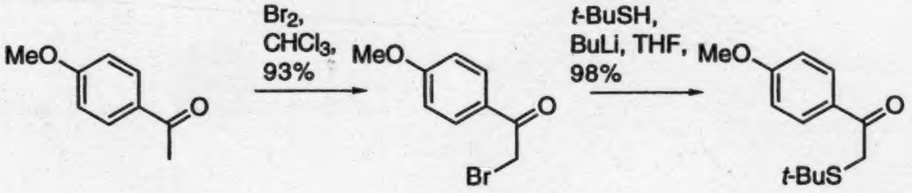
We started with the idea of reducing an imine along the lines shown in Scheme 2. An appropriate ketone (**3.3**) was made by the straightforward method summarized in Scheme 3, and proceeded without incident.


MeO
O
PgS
MeO
N
CO_2Pg'
R
PgS
MeO
H
N
CO_2Pg'
R
PgS

2.1 **2.2** **1.1**

Scheme 2

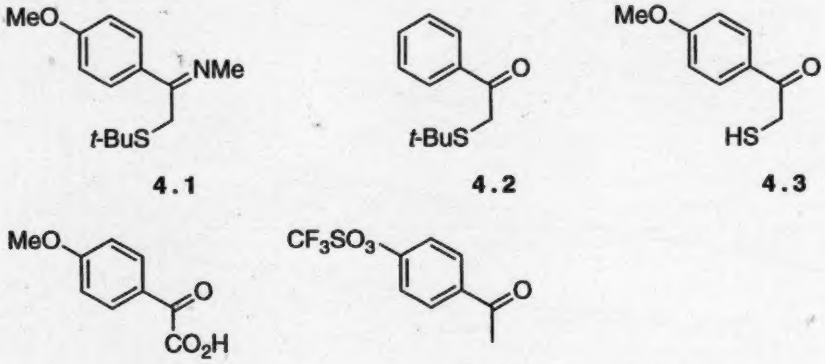
Bromo ketone **3.2** is a known compound,[1] and was prepared by addition of Br_2 to a solution of ketone **3.1**; displacement with *t*-BuSLi gave the desired thio ketone **3.3**.


MeO
O
Br_2,
$CHCl_3$,
93%
MeO
O
Br
t-BuSH,
BuLi, THF,
98%
MeO
O
t-BuS

3.1 **3.2** **3.3**

Scheme 3

Ketone **3.3** did not form an imine with $H_2NCH_2CO_2Me$ in the presence of either 4Å molecular sieves or of $HC(OMe)_3$, nor did it form an imine with $BnNH_2$ (also in presence of 4Å molecular sieves). A number of other attempts were made to generate imines. For example, we tried to prepare the simple imine **4.1**, using $TiCl_4$.[2] Our hope was that imine **4.1** would be more reactive than ketone **3.3** towards amines, and that any equilibrium would be driven to completion by expulsion of $MeNH_2$. Unfortunately, we were unable to prepare **4.1**.



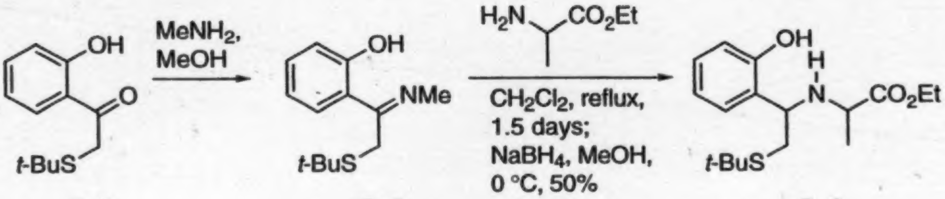
4.1 **4.2** **4.3**

4.4 **4.5**

Scheme 4

The possible effect of the methoxy group was then examined by attempting to form an imine between **4.2** and $H_2NCH_2CO_2Et$ (in the presence of 4Å molecular sieves); no reaction was observed. The related ketones **4.3**, **4.4**, and **4.5** were examined next. Ketone **4.3** did not react with $MeNH_2$ in MeOH, **4.4** did not react with $H_2NCH_2CO_2Et$ (4Å molecular sieves, $ClCH_2CH_2Cl$, MeOH, heat). Likewise, **4.5** failed to react with $H_2NCH_2CO_2Et$ ($MgSO_4$, CH_2Cl_2).

Finally, the *ortho*-hydroxy ketone **5.1** was examined, based on the fact that salicylaldehyde forms imines readily. Indeed, phenolic ketone **5.1** did form an imine with $MeNH_2$ (in



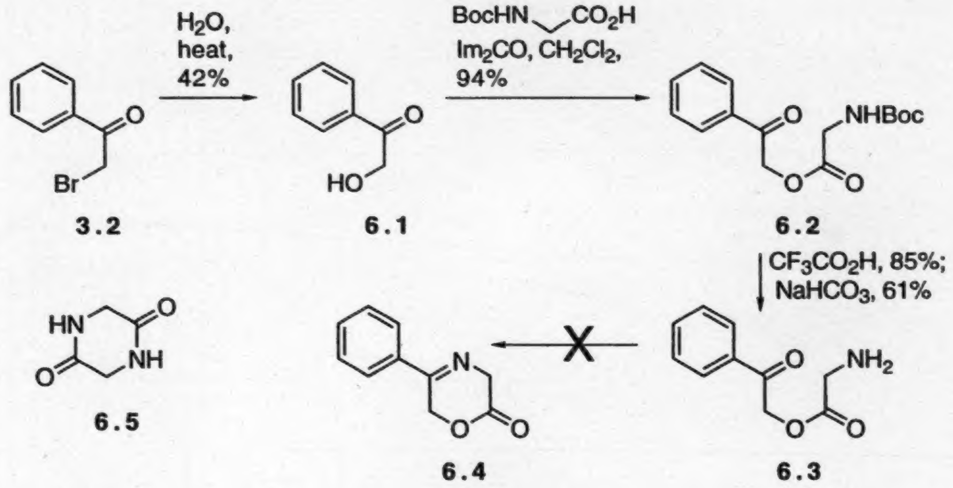
5.1 **5.2** **5.3**

Scheme 5

MeOH)[3] although, under the same conditions, (±)-$H_2NCHMeCO_2Et$ did not seem to react. Condensation with (±)-$H_2NCHMeCO_2Et$ was also tried in the presence of either 4Å molecular sieves (CH_2Cl_2 or $ClCH_2CH_2Cl$) or of a mixture of $MgSO_4$ and $Yb(OSO_2CF_3)_3$.[4] Imine **5.2** did react, although slowly (1.5 days in refluxing CH_2Cl_2), with (±)-$H_2NCHMeCO_2Et$ to give a new imine which was not isolated, but was reduced *in situ* ($NaBH_4$, MeOH). The phenolic amines **5.3** were isolated in 55% yield as a mixture of isomers. Our attempt to methylate the phenolic hydroxyl selectively (MeI, K_2CO_3, DMF) was not successful. We were, however, able to prepare the *O*-triflate, but could not remove the oxygen under standard conditions [$Pd(OAc)_2$, Ph_3P, Et_3N, HCO_2H, DMF).[5]

$B_{10}H_{14}$ is reported to catalyze both imine formation and subsequent reduction.[6] Unfortunately for us, it appears to be against US Federal Law to export even small research quantities and we were not able to obtain a sample, although, admittedly, we did not make extensive enquiries.

We turned next to explore the possibility of forming an imine by an intramolecular condensation.



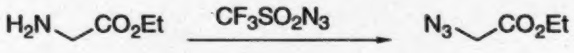
Scheme 6

Bromo ketone **3.2** was converted into the known hydroxy ketone **6.1**,[7] and this was coupled with $BocHNCH_2CO_2H$ to afford **6.2** in high yield (94%). Deprotection of the nitrogen and liberation of the resulting amine from the acid salt gave **6.3**. When this was heated in MeOH, **6.4** was not formed and, instead, the diketopiperazine **6.5** was isolated.

Aza-Wittig approach

We sought next to generate an aza-Wittig reagent by using the Staudinger reaction, in the hope that aza-Wittig reaction would afford an imine with ketone **3.3**. For this purpose, azide **7.2** was made from the corresponding amine by treatment with $CF_3SO_2N_3$.[8] Although the yield was very low (7%) in the single experiment carried out, we obtained enough material to generate the aza-Wittig reagent, by exposure of the azide to Ph_3P. This was done in the presence of ketone

3.3. However, no imine was formed. In a control experiment, Ph_3P was added to a mixture of acetophenone and $PhCH_2CH_2N_3$.


H2N CO2Et
CF3SO2N3
N3 CO2Et

7.1 **7.2**

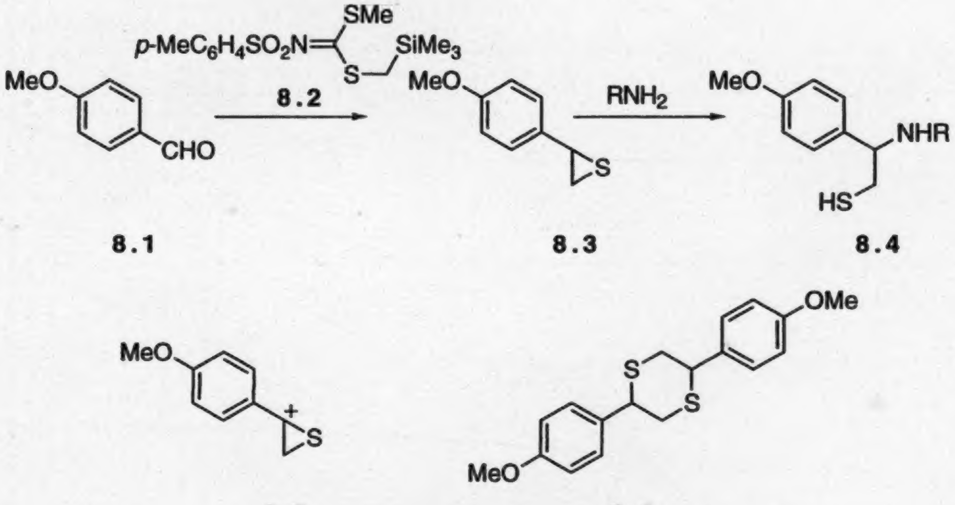
Scheme 7

The expected imine was not formed, and an unidentified product was isolated.

We also attempted to generate aza-Wittig reagents directly from $H_2NCH_2CO_2Et$ by treatment with Ph_3PBr_2 or with Ph_2MePCl_2,[9] but in neither case was the expected imine detected, and this approach was abandoned.

Approaches based on opening of episulfides

We considered that an episulfide such as **8.3** might react at the (activated) benzylic position with an amine (**8.3** →


SMe
p-MeC6H4SO2N=
SiMe3
S
MeO
CHO
8.2
MeO
S
RNH2
MeO
NHR
HS
MeO
+
S
OMe
S
S
MeO

8.1 **8.3** **8.4**

8.5 **8.6**

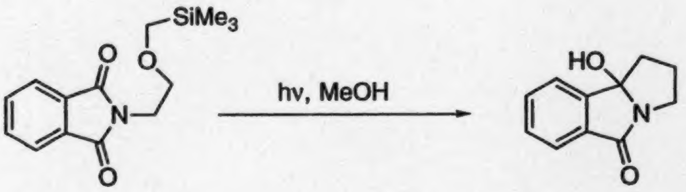
Scheme 8

8.4). Episulfide **8.3** is a known compound,[10] and was made from *p*-methoxybenzaldehyde (**8.1**) by treatment with **8.2**, as described in the literature. Treatment of **8.3** with $BnNH_2$, with or without the addition of $Hg(OCOCF_3)_2$ or Et_3B, caused destruction of the episulfide. When DDQ was used as the additive, in the hope of generating the species **8.5**, which would be captured by a primary amine, only the dimer **8.6** was isolated.

An attempt to alkylate episulfide **8.3** with BnBr gave no bromo sulfide, and treatment of **8.3** with $H_2NCH_2CO_2Me$ (DMF, room temperature) led to recovery of the episulfide.

Approaches based on photocyclization of an imine

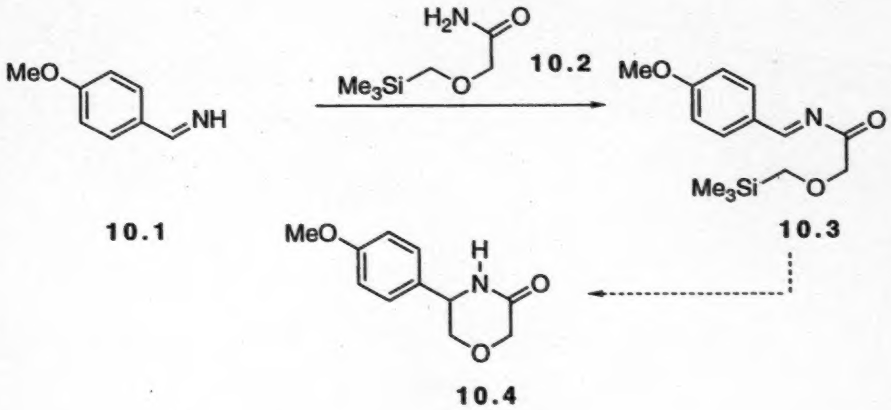
The report[11] that silane **9.1** undergoes photocyclization to **9.2** prompted us to attempt the cyclization of **10.3** to **10.4**.



9.1 **9.2**

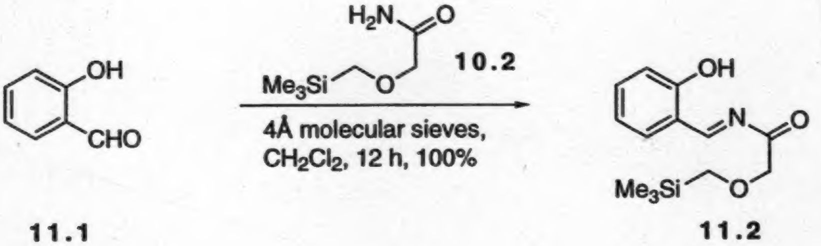
Scheme 9

Treatment of *p*-methoxybenzaldehyde with NH_3 in PhH, according to the literature procedure,[12] gave imine **10.1**. Condensation with **10.2**[13] in CH_2Cl_2 in the presence of 4Å molecular sieves then produced **10.3**, but this compound was unstable and was not examined further. The corresponding


MeO
NH
10.1
H_2N
O
Me_3Si
O
10.2
MeO
N
O
Me_3Si
O
10.3
MeO
H
N
O
O
10.4

Scheme 10

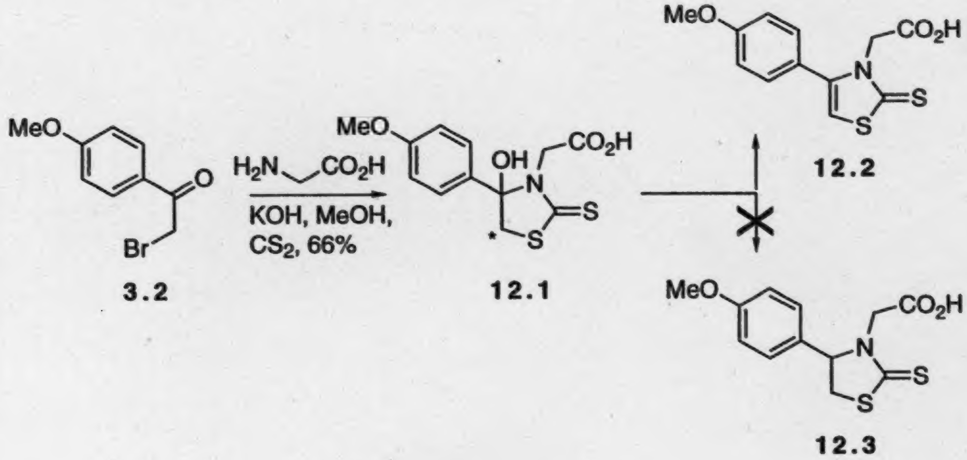
salicylaldehyde derivative **11.2**, was easily made (Scheme 11), but was inert to irradiation at 254 nm (medium pressure mercury lamp). Treatment of **11.2** with CsF in MeCN or with Bu_4NF in THF – in an attempt to effect ionic ring closure – gave none of the desired product, and only complex mixtures were obtained.


OH
CHO
11.1
H_2N
O
Me_3Si
O
10.2
4Å molecular sieves,
CH_2Cl_2, 12 h, 100%
OH
N
O
Me_3Si
O
11.2

Scheme 11

Dithiocarbamate approach

Following a general procedure reported for other bromo ketones,[15] **3.2** was converted into **12.1** by successive treatment with glycine, KOH and CS_2, but attempt to reductively remove the hydroxyl (**12.1** → **12.3**) ($NaBH_4$, MeOH or $BF_3.OEt_2$, Et_3SiH) gave instead the unsaturated compound **12.2** which, itself, was not reduced by diimide or by heating with HCO_2H-Et_3SiH. A way to avoid

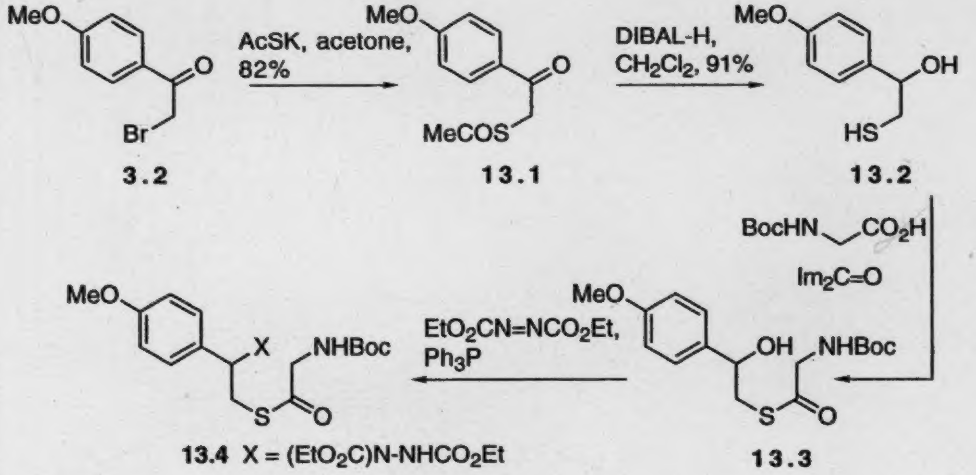


Scheme 12

formation of the double bond would be to prepare an analog of **12.1** in which the starred atom carries two methyl groups, but our later experiments (see page 223) showed that the heterocyclic system cannot be opened under sufficiently mild conditions that a stereogenic center on the amino acid would not be epimerized.

Intramolecular Mitsunobu approach

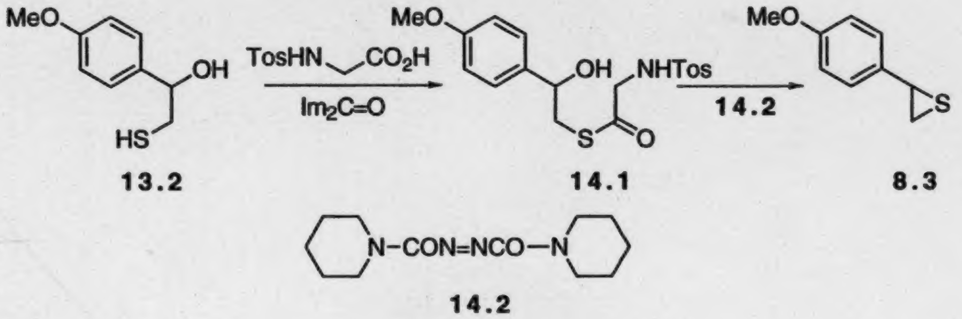
The hydroxy thiol **13.2**, readily made from the known ketone **13.1**,[16] as shown in Scheme 13, was acylated with $BocHNCH_2CO_2H$ to afford **13.3**. Under the conditions of the Mitsunobu reaction, the nitrogen failed to close onto the



Scheme 13

benzylic carbon and, instead, the adduct **13.4** was obtained.

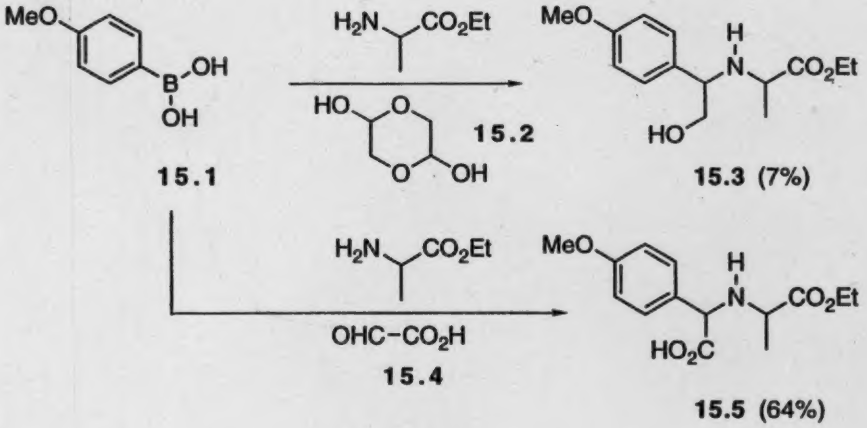
When we tried the corresponding experiment with the *N*-tosyl thioester **14.1**, using a modified version of the Mitsunobu process,[17] episulfide **8.3** was obtained and again there was no cyclization through nitrogen.



Scheme 14

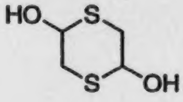
Boronic acid approach

Boronic acids have been reported[18] to react with α-hydroxy- or α-carboxy aldehydes in the presence of amines to generate β-amino alcohols, and we attempted to apply this methodology to our own problem. To this end, boronic acid **15.1** was treated with glycol aldehyde dimer (**15.2**) and (±)-$H_2NCHMeCO_2Et$. The expected product **15.3** was indeed obtained, but only in 7% yield. When glyoxylic acid (**15.4**) was used instead, **15.5** was obtained in 64% yield. However, an attempt to reduce the carbonyl selectively, using $BH_3.SMe_2$, AcOH, I_2, MeOH,[19] did not give the required alcohol.



Scheme 15

An attempt to make the known compound **16.1**[20] was unsuccessful; had we obtained it, we would have used it with



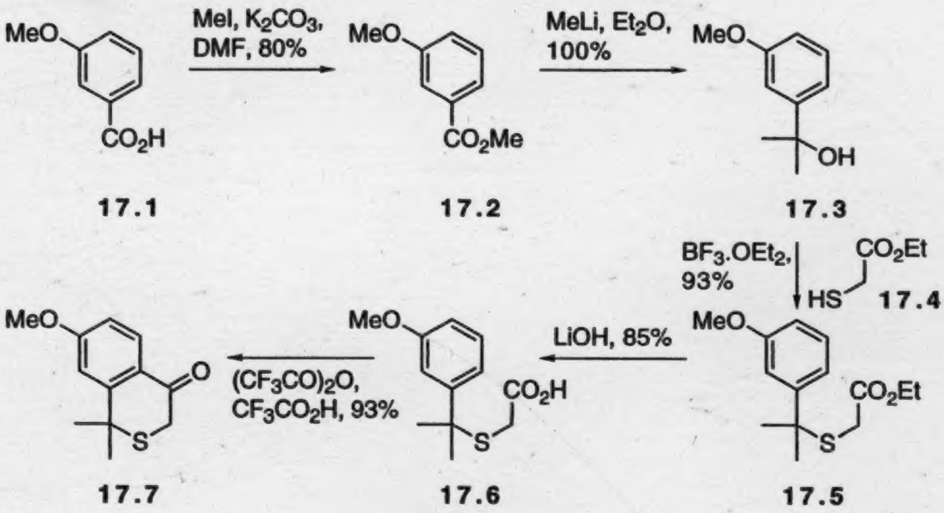
16.1

Scheme 16

boronic acid **15.1** in place of **15.2** (see Scheme 15). Before we had an opportunity to identify what had caused the preparation of **16.1** to fail in our hands, a successful route to compounds of type **1.1** was identified and so no further work was done with boronic acids.

Cyclic sulfide approach

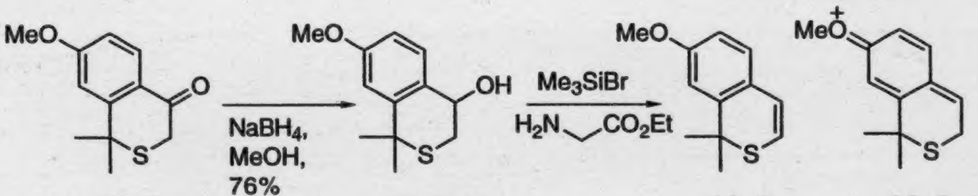
Some of the problems we had encountered might have been caused by steric factors introduced by the *t*-BuS group, and we next sought to avoid such difficulties by incorporating the sulfur into a ring, as in **17.7** (Scheme 17).


MeO
CO2H
17.1
MeI, K2CO3, DMF, 80%
MeO
CO2Me
17.2
MeLi, Et2O, 100%
MeO
OH
17.3
BF3.OEt2, 93%
CO2Et
HS
17.4
MeO
CO2Et
S
17.5
LiOH, 85%
MeO
CO2H
S
17.6
(CF3CO)2O, CF3CO2H, 93%
MeO
O
S
17.7

Scheme 17

m-Methoxybenzoic acid (**17.1**) was methylated (Scheme 17) and the resulting ester was converted into the tertiary alcohol **17.3** by the action of MeLi. Condensation with thiol ester **17.4**,[21] mediated by $BF_3.OEt_2$, gave the sulfide **17.5**, from which point ester hydrolysis and cyclization afforded the desired ketone **17.7**. All the yields in this sequence were high.

With ketone **17.7** in hand, we tried to form an imine with (±)-$H_2NCHMeCO_2Et$ (PhH, TsOH.H_2O, heat), but no reaction occurred.

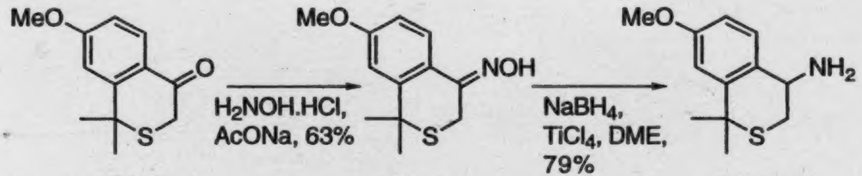

MeO
O
S
NaBH$_4$,
MeOH,
76%
MeO
OH
S
Me$_3$SiBr
H$_2$N⌄CO$_2$Et
MeO
S
MeO$^+$
S

17.7 **18.1** **18.2** **18.3**

Scheme 18

Reduction of ketone **17.7** (NaBH$_4$, MeOH, 76%) gave alcohol **18.1**. This was treated with Me_3SiBr and $H_2NCH_2CO_2Et$ with the intention of generating the corresponding bromide or a quinone methide intermediate (see **18.3**); either species would then be trapped by the amine. In the event, however, the experiment gave **18.2**.

Ketone **17.7** was readily converted into oxime **19.1**, and reduction with NaBH$_4$-TiCl$_4$[22] gave amine **19.2**, which we planned to use for displacement of halogen from chiral α-

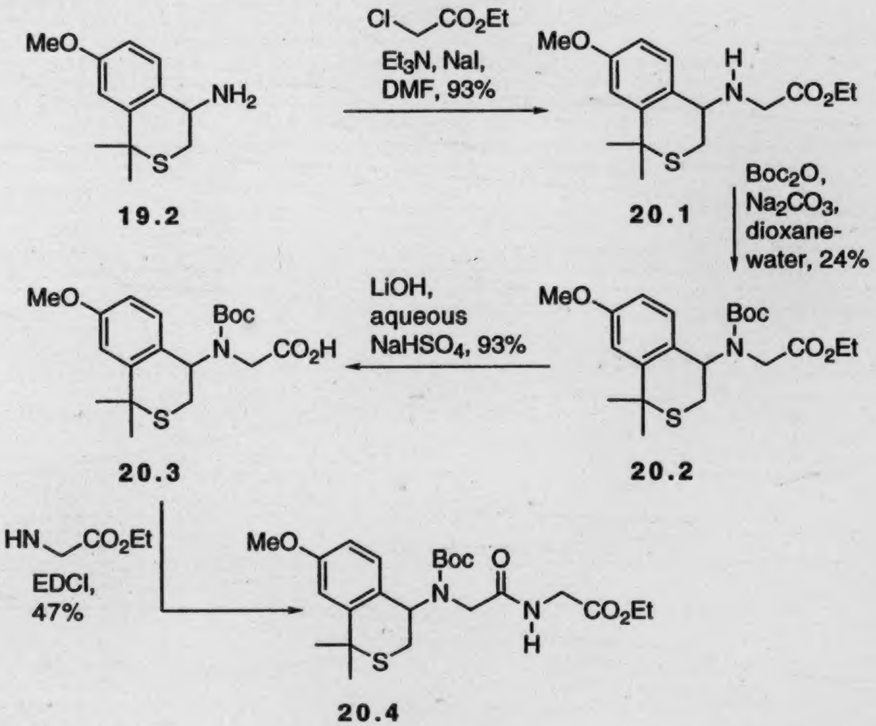

MeO
O
S
H$_2$NOH.HCl,
AcONa, 63%
MeO
NOH
S
NaBH$_4$,
TiCl$_4$, DME,
79%
MeO
NH$_2$
S

17.7 **19.1** **19.2**

Scheme 19

bromo esters – a process closely related to Kent's approach.[23]

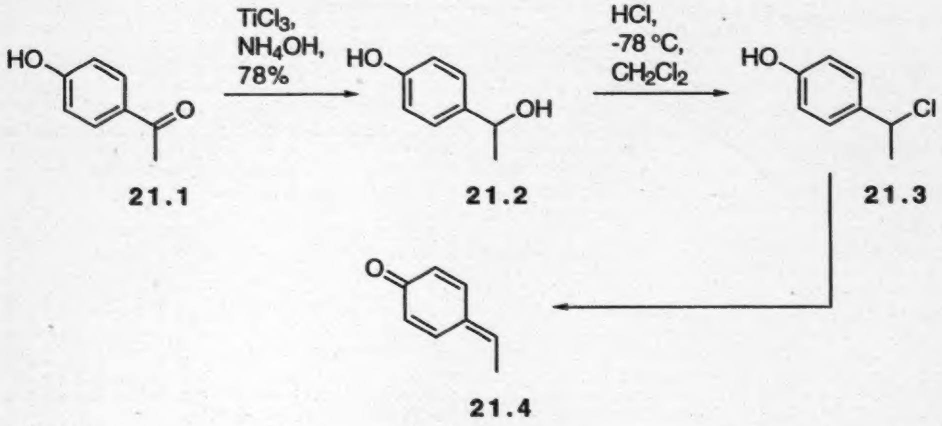
Amine **19.2** reacted readily with $ClCH_2CO_2Et$ to give **20.1** (Scheme 20). Protection of the nitrogen as a carbamate and selective hydrolysis of the ethyl ester took the route to **20.3**, at which point we coupled the acid with $H_2NCH_2CO_2Et$. The next task was to release a thiol group in **20.4**. This step was tried with the simple model **20.1**. Surprisingly, the compound was stable to the standard conditions [$Hg(OAc)_2$, CF_3CO_2H] even with a prolonged reaction time (2 days). Consequently, this route was abandoned.


MeO
NH2
19.2
Cl CO2Et
Et3N, NaI,
DMF, 93%
MeO
H
N CO2Et
20.1
Boc2O,
Na2CO3,
dioxane-
water, 24%
MeO
Boc
N CO2Et
20.2
LiOH,
aqueous
NaHSO4, 93%
MeO
Boc
N CO2H
20.3
HN CO2Et
EDCI,
47%
MeO
Boc
O
N
H
CO2Et
20.4

Scheme 20

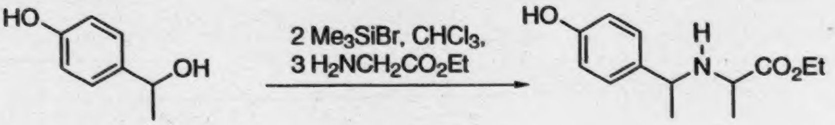
Quinone methide and related approaches — a successful route

We decided at this point to investigate the possibility of adding an amine (as in $H_2NCH_2CO_2R$) to a quinone methide and, in the event, this approach led to an acceptable route to our target compounds of type **1.1**.



Scheme 21

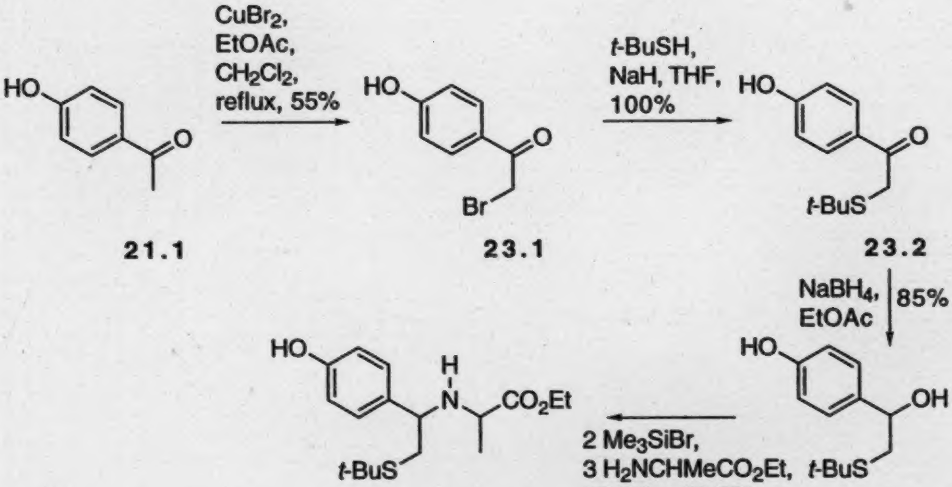
The quinone methide **21.4** has been prepared, but not isolated from its solution.[24] We reduced ketone **21.1** to alcohol **21.2** using $TiCl_3$ in NH_4OH-MeOH.[25] Use of $NaBH_4$ failed to give **21.2**. With **21.2** in hand, treatment with HCl and then with Et_3N, as reported in the literature,[24] gave a solution of **21.4**, but removal of the solvent resulted in decomposition. Accordingly, we decided to generate the quinone methide *in situ*. It was more convenient for us to make the bromide corresponding to **21.3** (by using Me_3SiBr[26]), and we also decided to employ an excess of amine[27] to effect elimination of HBr. Treatment of **21.2** in dry $CHCl_3$ with 2


2 Me3SiBr, CHCl3,
3 H2NCH2CO2Et

21.2 **22.1**

Scheme 22

equivalents of Me_3SiBr (there are 2 hydroxyl groups in **21.2**), followed by addition of 3 equivalents[28] of (±)-$H_2NCHMeCO_2Et$ gave **22.1**. Based on this promising result, we then set out to try a similar reaction with a protected sulfur unit in place.

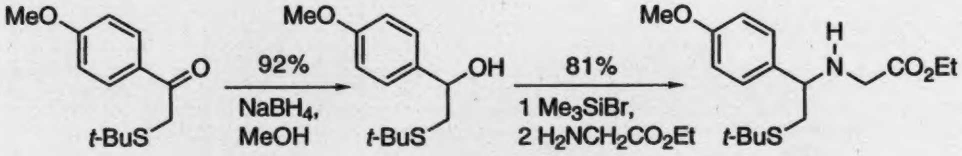

CuBr2,
EtOAc,
CH2Cl2,
reflux, 55%
t-BuSH,
NaH, THF,
100%
NaBH4,
EtOAc
85%
2 Me3SiBr,
3 H2NCHMeCO2Et,
29.6% + 29.7%

21.1 **23.1** **23.2** **23.3** **23.4**

Scheme 23

Phenolic ketone **21.1** was converted into bromide **23.1**[29] by the action of $CuBr_2$, as described in the literature (Scheme 23). Displacement of bromide with *t*-BuSNa and reduction ($NaBH_4$) gave the required alcohol **23.3**. When this was treated with 2 equivalents of Me_3SiBr and 3 equivalents of (±)-$H_2NCHMeCO_2Et$ the desired amine **23.4** was obtained as a separable mixture of diastereoisomers.

During the optimization of this reaction it was noticed that the use of only 1 equivalent of Me_3SiBr and 2 equivalents of (±)-$H_2NCHMeCO_2Et$ was just as effective as the use of 2 and 3 equivalents, respectively. This suggested that the reaction might not occur through a quinone methide (which would require 3 equivalents of amine), and for this reason, we examined the methoxy series, as summarized in Scheme 24. When only 1 equivalent of $H_2NCH_2CO_2Et$ was used, together with 1 equivalent of a sacrificial base (*i*-Pr_2NEt), a lower yield (ca 37%) of **24.2** was obtained.



3.3 **24.1** **24.2**

Scheme 24

Surprisingly, in the reactions with Me_3SiBr neighboring group participation by the sulfur with loss of the *t*-butyl group does not occur.

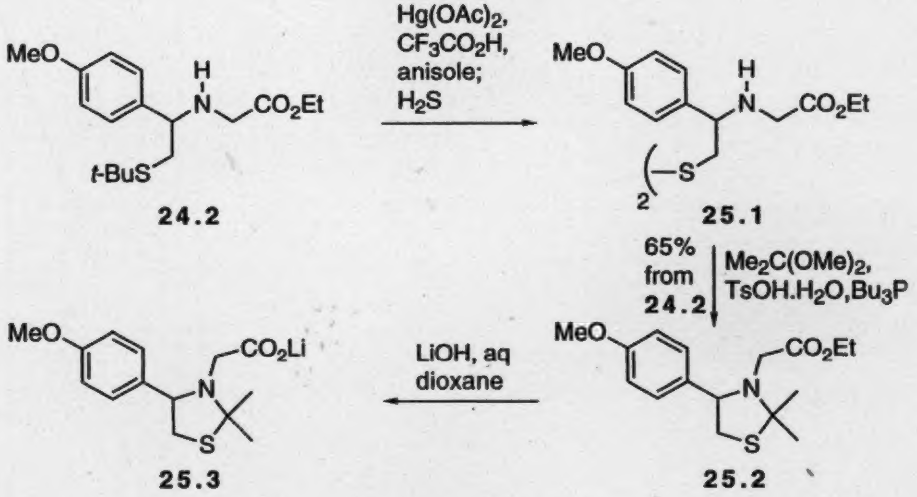
With a method for making compounds of type **24.2** in hand, we considered a number of procedures for protection of the nitrogen and sulfur atoms and the carboxyl group.

Studies on nitrogen and sulfur protection

We first investigated the possibility of protecting the nitrogen and sulfur in such a way that *both* heteroatoms could be deprotected in a single step, after our derivatized amino acid (cf. **1.1**) had been incorporated as the *N*-terminus of a peptide segment.

Thiazolidine route for simultaneous protection of nitrogen and sulfur

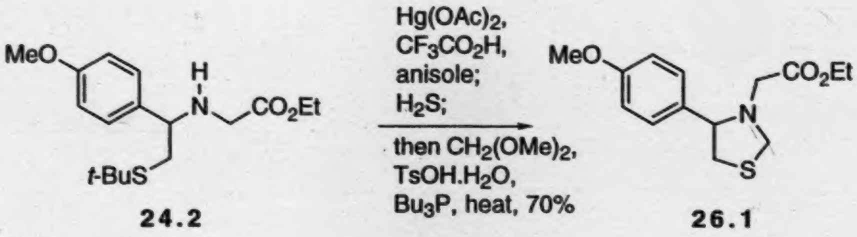
Treatment of **24.2** with $Hg(OAc)_2$ in CF_3CO_2H containing $7.5^v/_v\%$ anisole, followed by addition of H_2S, and exposure to air gave **25.1**. This was converted into the thiazolidine **25.2** by heating in $Me_2C(OMe)_2$ in the presence of $TsOH.H_2O$ and Bu_3P – the latter added in order to reduce the disulfide. Hydrolysis of the ester group with LiOH produced the lithium salt **25.3**, but he free carboxylic acid could not be obtained


MeO
H
N
CO2Et
t-BuS
24.2
Hg(OAc)2,
CF3CO2H,
anisole;
H2S
MeO
H
N
CO2Et
S
2
25.1
65%
from
24.2
Me2C(OMe)2,
TsOH.H2O,Bu3P
MeO
CO2Et
N
S
25.2
LiOH, aq
dioxane
MeO
CO2Li
N
S
25.3

Scheme 25

by acidification to pH 2-3, and we gained the impression that in the carboxylic acid form the heterocycle is very acid-sensitive.

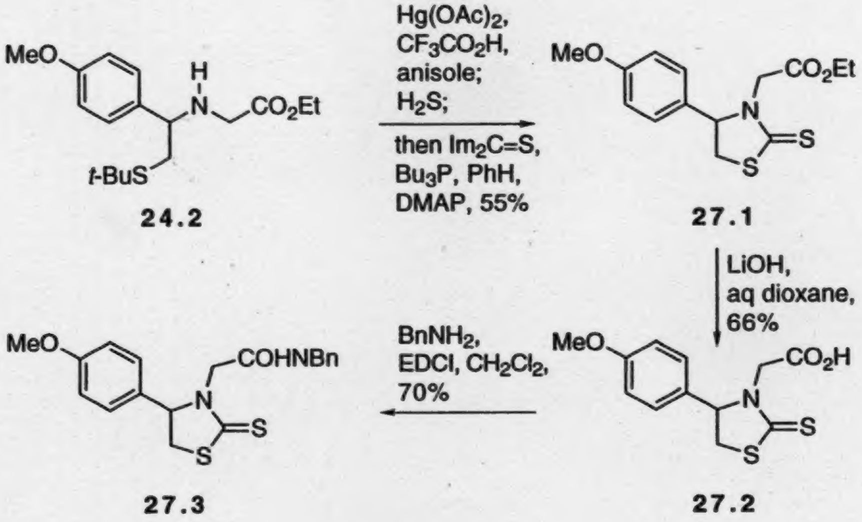
The lithium salt did not form an amide on attempted coupling with $BnNH_2$ in the presence of EDCI.[30] When the lithium salt was quenched with 1 equivalent of HCl, treatment with $BnNH_2$ and EDCI again failed to give the coupled amide. We therefore examined an unsubstituted thiazolidine in the expectation that it would be more acid-stable. Accordingly, the *t*-butyl group of **24.2** was removed in the usual way and the crude product was converted into the thiazolidine **26.1** by using $CH_2(OMe)_2$ under conditions of acid catalysis. Hydrolysis of the ester with LiOH in aqueous dioxane resulted in opening of the heterocycle, even without acidification.



Scheme 26

Thiocarbamate route for simultaneous protection of nitrogen and sulfur

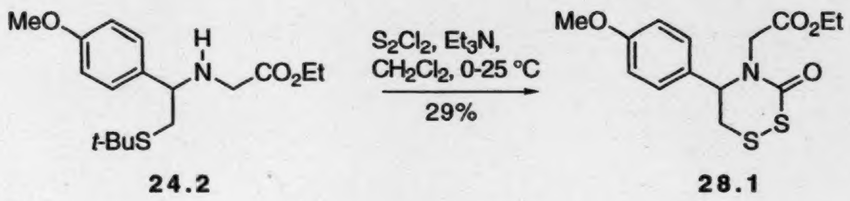
Deprotection of sulfur in **24.2** and reaction with $Im_2C{=}S$ gave the thiocarbamate **27.1** in 55% yield. Hydrolysis of the ester and coupling with $BnNH_2$ (EDCI) gave **27.3**. At this point we were not able to deprotect the nitrogen and sulfur. Treatment with $Hg(OAc)_2$ in aqueous MeCN led to recovery of starting material, and use of $Hg(OAc)_2$ in CF_3CO_2H gave a product that was not properly characterized, but which is probably the C=O analog of **27.3** ([C=O instead of C=S), based on slight chemical shift differences between **27.3** and the new compound.


MeO
H
N
CO2Et
t-BuS
24.2
Hg(OAc)2, CF3CO2H, anisole; H2S;
then Im2C=S, Bu3P, PhH, DMAP, 55%
27.1
LiOH, aq dioxane, 66%
CO2H
27.2
BnNH2, EDCl, CH2Cl2, 70%
COHNBn
27.3
S

Scheme 27

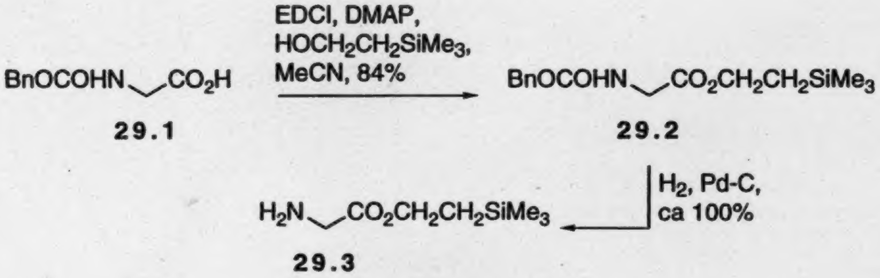
Dithiocarbamoyl route for simultaneous protection of nitrogen and sulfur

Compound **24.2** reacted with S_2Cl_2 to give **28.1** (Scheme 28), but exposure to LiOH produced a complex mixture. Clearly, **28.1** is too sensitive to base, and so we prepared the corresponding β-(trimethylsilyl)ethyl ester with the


MeO
t-BuS
CO$_2$Et
S_2Cl_2, Et_3N,
CH_2Cl_2, 0-25 °C
29%
24.2
28.1

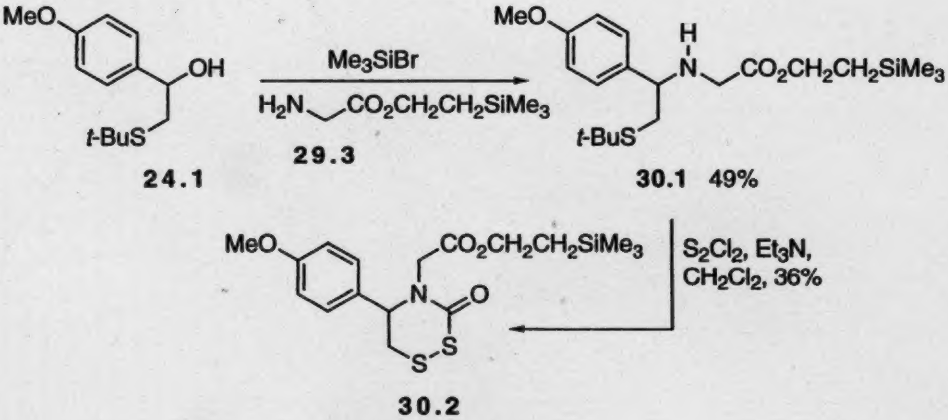
Scheme 28

intention of removing the ester group by the action of fluoride ion.[31]


EDCI, DMAP,
$HOCH_2CH_2SiMe_3$,
MeCN, 84%
$BnOCOHN\smile CO_2H$
$BnOCOHN\smile CO_2CH_2CH_2SiMe_3$
29.1
29.2
H_2, Pd-C,
ca 100%
$H_2N\smile CO_2CH_2CH_2SiMe_3$
29.3

Scheme 29

The requisite ester **29.3**[32] was made in the straightforward way summarized in Scheme 29 by coupling


MeO
OH
t-BuS
24.1
Me_3SiBr
$H_2N\smile CO_2CH_2CH_2SiMe_3$
29.3
$CO_2CH_2CH_2SiMe_3$
30.1 49%
S_2Cl_2, Et_3N,
CH_2Cl_2, 36%
30.2

Scheme 30

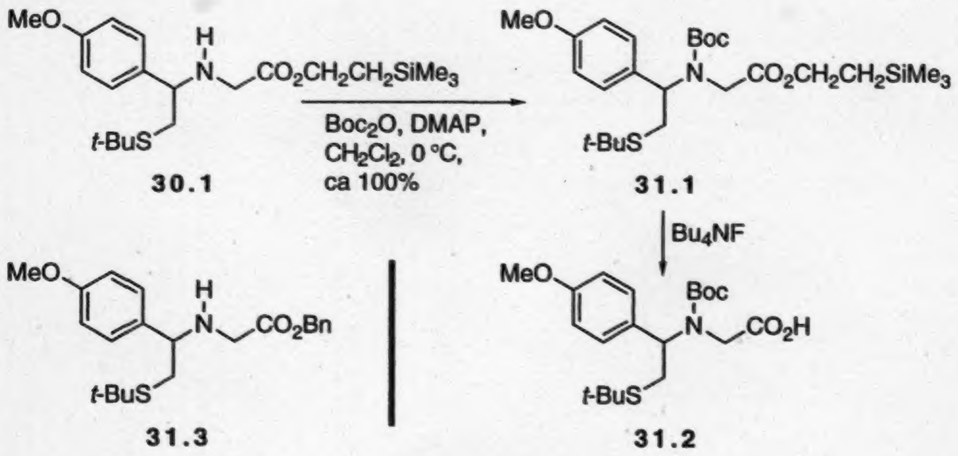
$BnOCOHNCH_2CO_2H$[33] with $HOCH_2CH_2SiMe_3$,[21] and deprotecting the nitrogen. The compound dimerizes even when stored at -20 °C and so freshly prepared material must be used.

Alcohol **24.2** was then treated with Me_3SiBr (1 equivalent) and 2 equivalents of **29.3** to obtain **30.1** (49%). Reaction with S_2Cl_2 gave **30.2**, but this produced a complex mixture when treated with Bu_4NF.

At this stage attempts to protect both nitrogen and sulfur simultaneously were abandoned.

Carboxyl protection as a β-(trimethylsilyl)ethyl ester

Ester **30.1** was protected on nitrogen, using Boc_2O to form **31.2** (Scheme 31) in almost quantitative yield.

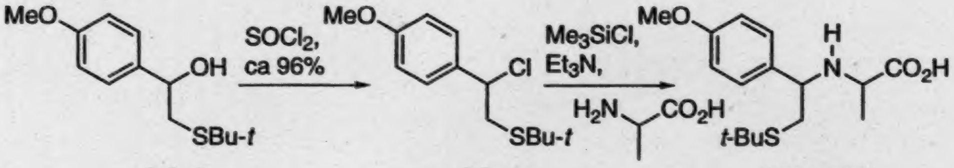

MeO
H
N
$CO_2CH_2CH_2SiMe_3$
t-BuS
30.1
Boc_2O, DMAP,
CH_2Cl_2, 0 °C,
ca 100%
Boc
31.1
Bu_4NF
CO_2Bn
31.3
CO_2H
31.2

Scheme 31

Treatment with Bu_4NF appeared (^1H NMR) to give the desired acid **31.2**, but the material decomposed on attempted chromatography.

The benzyl ester **31.3** (made in the same way as **30.1**) was recovered unchanged on attempted hydrogenolysis (H_2, Pd-C or Pd-$BaSO_4$, EtOH), and so we decided to examine the trichloroethyl ester series. However, **24.1** did not afford the desired amine when treated with Me_3SiBr and $H_2NCH_2CO_2CH_2CCl_3$.[34]

We eventually settled on the ethyl ester for glycine and the *t*-butyl ester for alanine and serine – the three amino acids we investigated in our work. Use of a base with esters other than glycine might cause epimerization; *t*-butyl esters can be hydrolyzed under acidic conditions and are resistant to nucleophilic attack. We did, however, examine briefly the possibility of avoiding carboxyl protection altogether (Scheme 32). To this end, alcohol **24.1** was converted into its chloride, which was then treated with the silyl ester formed by refluxing a chloroform solution of Me_3SiCl, (±)-alanine, and Et_3N.[35] This procedure gave **32.2**, after aqueous acidic workup. Although we do obtain the desired product we decided not to use this route because of the long reaction time in the presence of the organic base; this might compromise the stereochemical integrity of the amino acid – a feature that was not examined in this experiment.



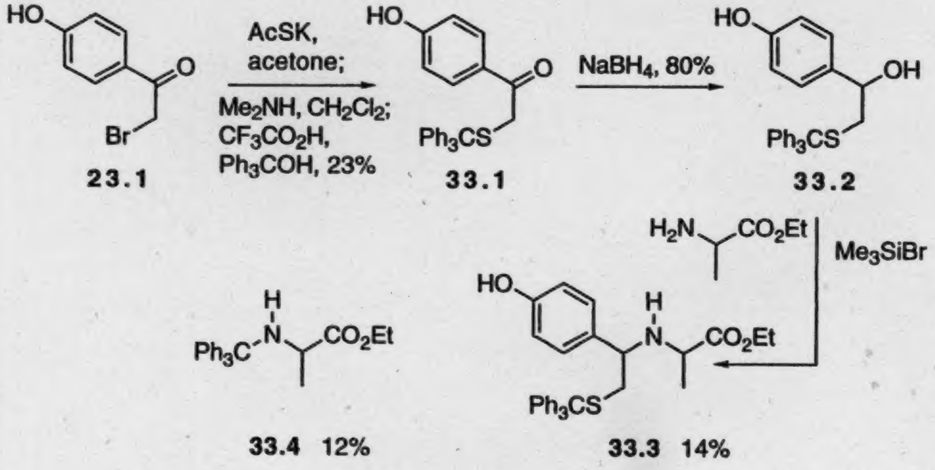
24.1 **32.1** **32.2** 31%

Scheme 32

Sulfur protection

Besides the *t*-butyl group, we also examined two other methods for protecting the sulfur.

Before we had settled on methoxy substitution of the benzene ring, the *S*-trityl series shown in Scheme 33 was examined. Bromide **23.1** was converted by the standard reactions shown into the *S*-trityl ketone **33.1**. Reduction gave alcohol **33.2**, and this was coupled by our Me_3SiBr method with (±)-$H_2NCHMeCO_2Et$. Although he desired trityl-protected derivative **33.3** was obtained, the yield was low; there was appreciable transfer of the trityl group to nitrogen, and compound **33.4** was isolated in 12% yield.

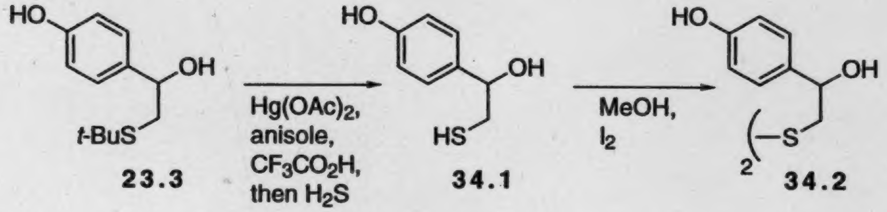


Scheme 33

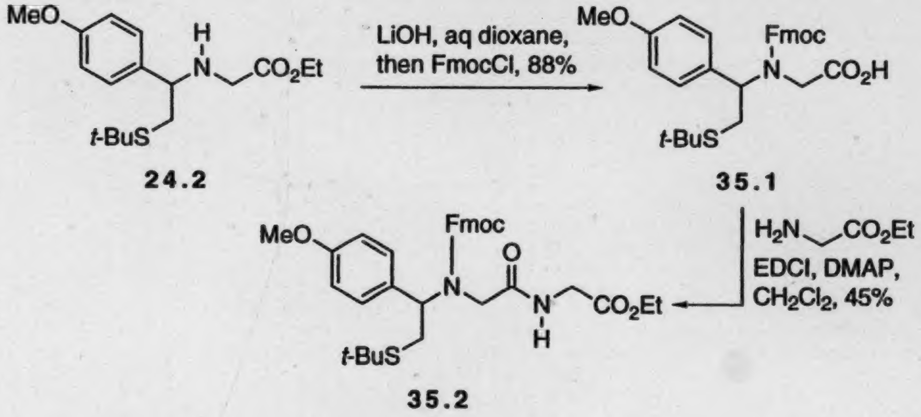
We also took alcohol **23.3**, removed the *t*-butyl group (**23.3** → **34.1**) and oxidized the product to its disulfide **34.2**, but this substance failed to undergo coupling with (±)-$H_2NCHMeCO_2Et$ in the presence of Me_3SiBr.

Nitrogen protection

The derivatized amino acid **24.2** was hydrolyzed with LiOH in aqueous dioxane and the mixture was quenched by addition of FmocCl to obtain **35.1** in 88% yield. The acid was then coupled with $H_2NCH_2CO_2Et$, but an attempt to remove the Fmoc group from the product (**35.2**) by treatment with Et_2NH gave unidentified products. A similar sequence was performed using Boc_2O instead of FmocCl (Scheme 36).

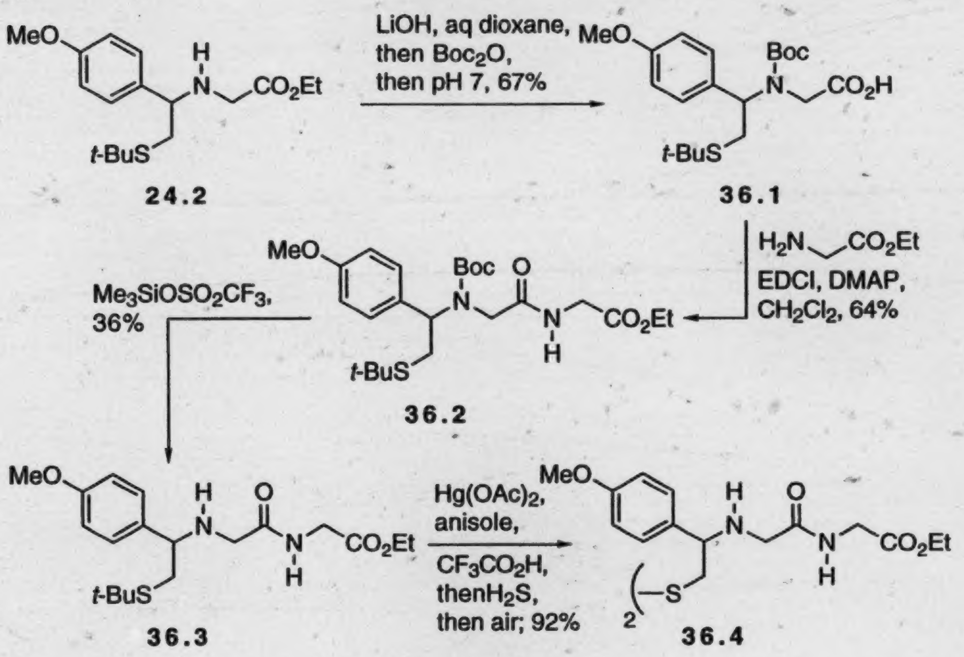

HO
OH
t-BuS
23.3
Hg(OAc)2, anisole, CF3CO2H, then H2S
HS
34.1
MeOH, I2
S
2
34.2

Scheme 34


MeO
H
N
CO2Et
t-BuS
24.2
LiOH, aq dioxane, then FmocCl, 88%
Fmoc
CO2H
35.1
H2N CO2Et
EDCI, DMAP, CH2Cl2, 45%
O
35.2

Scheme 35

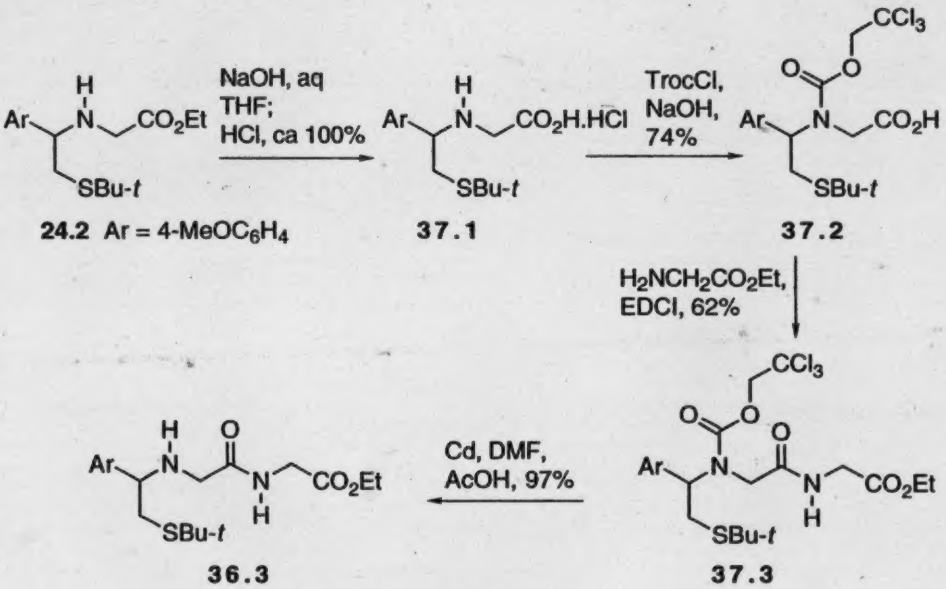
The *N*-Boc acid **36.1**, could not be purified by flash chromatography, although a cyclohexylamine salt could be made. Coupling with $H_2NCH_2CO_2Et$ gave **36.2**. Removal of the *N*-Boc group required extensive experimentation,[36] until we tried $Me_3SiOSO_2CF_3$, an experiment that gave the expected amine


MeO
H
N
CO2Et
t-BuS
24.2
LiOH, aq dioxane,
then Boc2O,
then pH 7, 67%
Boc
CO2H
36.1
H2N CO2Et
EDCI, DMAP,
CH2Cl2, 64%
Boc O
36.2
Me3SiOSO2CF3,
36%
36.3
Hg(OAc)2,
anisole,
CF3CO2H,
thenH2S,
then air; 92%
S
2
36.4

Scheme 36

36.3. Removal of the *S*-protecting group and oxidation, best done by exposure to air rather than by use of I_2-MeOH, gave disulfide **36.4**. Because of the low yield in the removal of the Boc group we subsequently used Troc [trichloroethyl carbamate, $Cl_3CCH_2OC(O)$] protection (Scheme 37).

Ester **24.2** was hydrolyzed with aqueous base, and the nitrogen was protected as its Troc carbamate under standard conditions.[37] Coupling with $H_2NCH_2CO_2Et$ gave **37.3** and the Troc group was removed with Cd in DMF-AcOH.[38] As described above (Scheme 36), the sulfur protecting group of compound



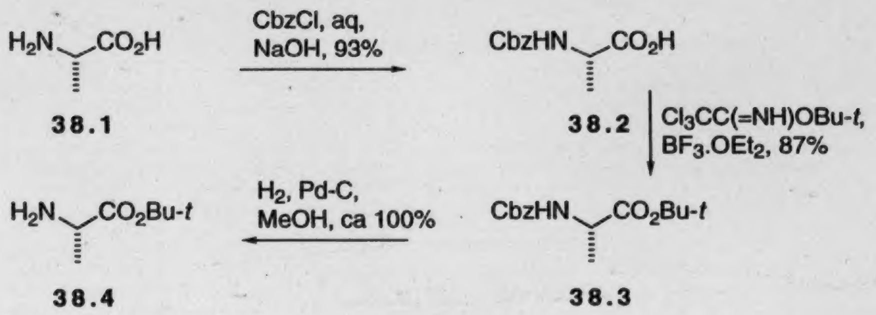
NaOH, aq
THF;
HCl, ca 100%
TrocCl,
NaOH,
74%
H2NCH2CO2Et,
EDCI, 62%
Cd, DMF,
AcOH, 97%
24.2 Ar = 4-MeOC6H4
37.1
37.2
37.3
36.3

Scheme 37

36.3 can be removed efficiently. The yields in this sequence were acceptable and the route represents our optimized version of making a specially derivatized glycine of the type **1.1**. Coupling of **37.2** with $H_2NCH_2CO_2Et$ was done in order to show that deprotection of both nitrogen and sulfur could be accomplished in a situation that resembles the one that would be present when the derivatized amino acid is the *N*-terminus of a peptide.

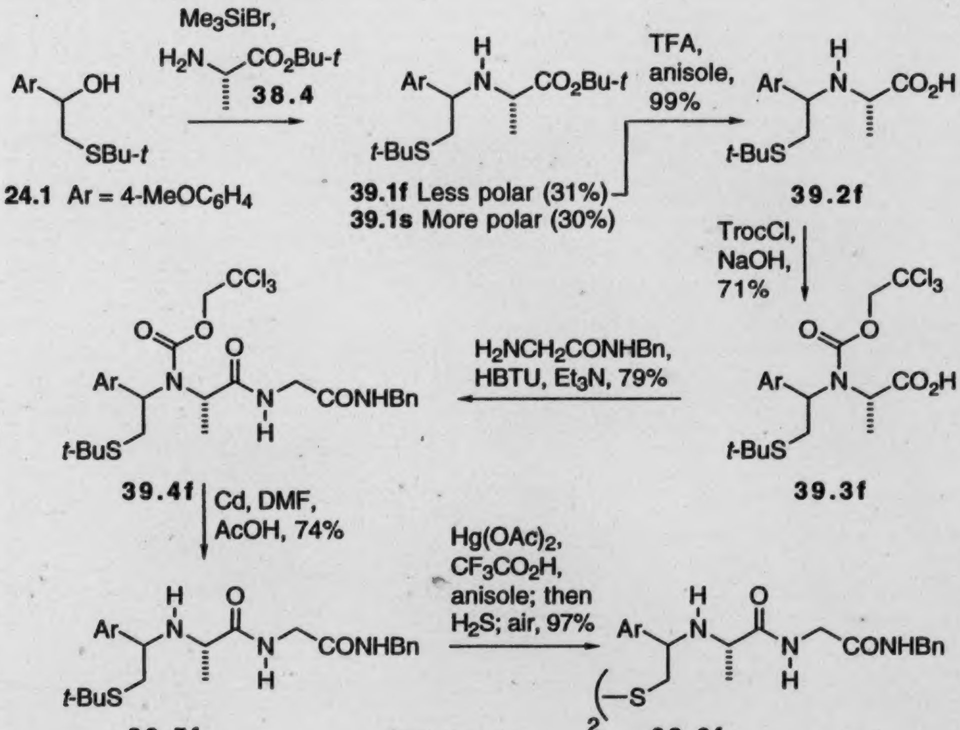
We next repeated the sequence using L-alanine, for which we needed L-alanine *t*-butyl ester (**38.4**). The preparation of this known ester[39,40] was initially troublesome, but we eventually found a route that gives the product without epimerization (Scheme 38).

L-Alanine was protected as its Cbz carbamate (**38.2**),[33]


H2N CO2H
38.1
CbzCl, aq, NaOH, 93%
CbzHN CO2H
38.2
Cl3CC(=NH)OBu-t, BF3.OEt2, 87%
CbzHN CO2Bu-t
38.3
H2, Pd-C, MeOH, ca 100%
H2N CO2Bu-t
38.4

Scheme 38

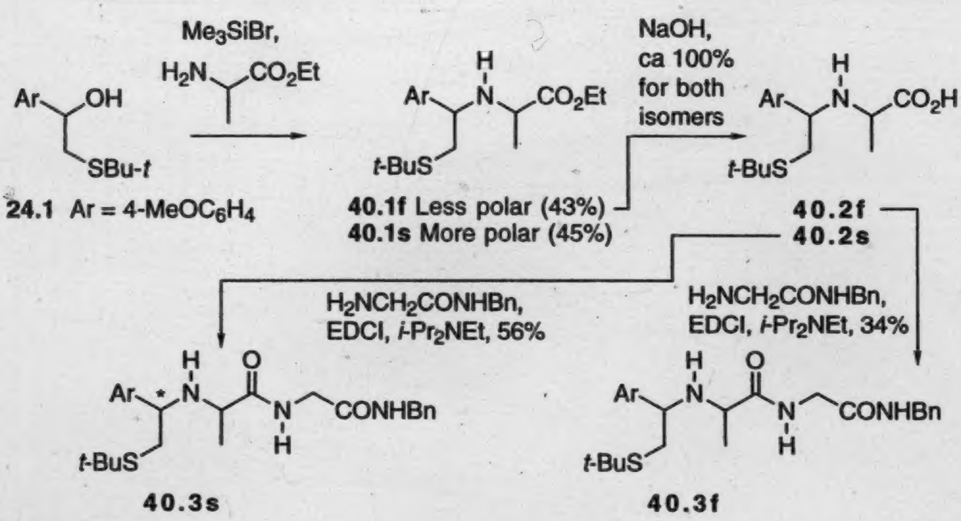
and the *t*-butyl ester **38.3** was prepared using the *t*-butyl trichloroacetimidate reagent.[41] Hydrogenolysis then gave L-alanine *t*-butyl ester (**38.4**). The Mosher amide was prepared and found to give a single peak in the ^{19}F NMR spectrum. In


Ar OH
SBu-t
24.1 Ar = 4-MeOC6H4
Me3SiBr,
H2N CO2Bu-t
38.4
Ar N CO2Bu-t
H
t-BuS
39.1f Less polar (31%)
39.1s More polar (30%)
TFA, anisole, 99%
Ar N CO2H
t-BuS
39.2f
TrocCl, NaOH, 71%
CCl3
O O
Ar N CO2H
t-BuS
39.3f
H2NCH2CONHBn, HBTU, Et3N, 79%
Ar N N CONHBn
O
H
t-BuS
39.4f
Cd, DMF, AcOH, 74%
39.5f
Hg(OAc)2, CF3CO2H, anisole; then H2S; air, 97%
S
2
39.6f

Scheme 39

this case we did not examine the Mosher amides of racemic material but we did so later on when we investigated the serine series.

Alcohol **24.1** was coupled with the L-alanine *t*-butyl ester **38.4** under our optimized conditions to afford a separable mixture of the less polar (**39.1f**, 31%) and more polar (**39.1s**, 30%) adducts, respectively. The less polar isomer was hydrolyzed and protected by treatment with TrocCl (71%) and then coupled with $H_2NCH_2CONHBn$. Removal of the Troc protecting group (Cd, DMF, AcOH, 74%) and deprotection of the sulfur [$Hg(OAc)_2$, CF_3CO_2H, anisole, H_2S), followed by aerial oxidation, gave the expected disulfide **39.6f**. Compound **39.5f** has been sent to Boehringer Ingelheim (Laval) together with a sample made from racemic alanine for examination on a chiral column. We hope that the chromatographic tests will show that **39.5f** is a single enantiomer. The material made

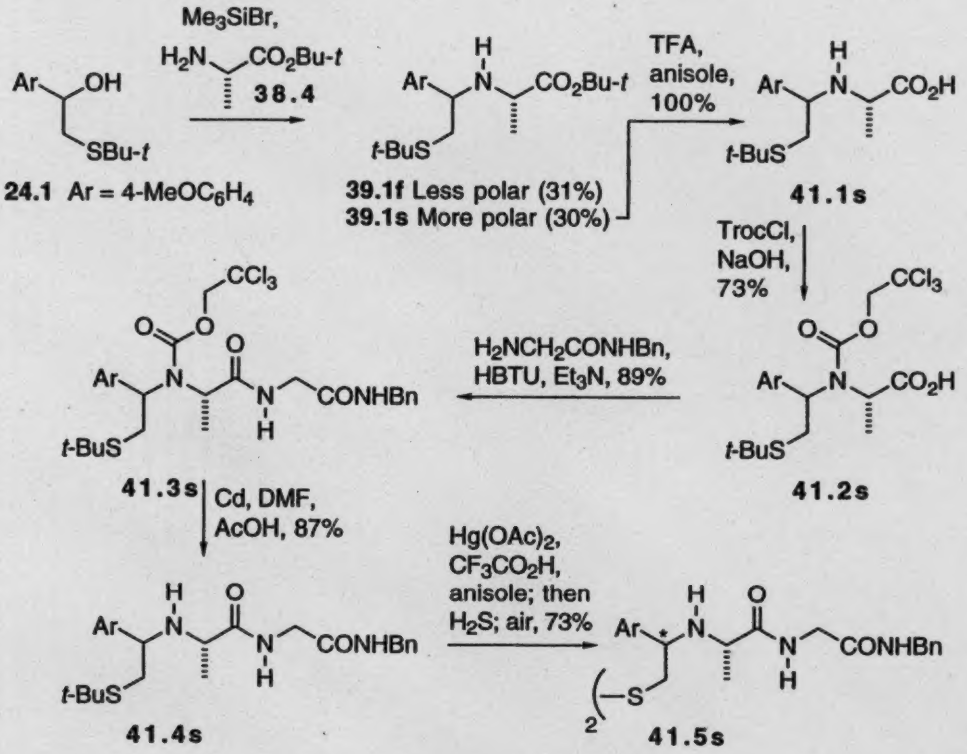

Me3SiBr,
H2N CO2Et
Ar OH
SBu-t
24.1 Ar = 4-MeOC6H4
H
Ar N CO2Et
t-BuS
40.1f Less polar (43%)
40.1s More polar (45%)
NaOH,
ca 100%
for both
isomers
H
Ar N CO2H
t-BuS
40.2f
40.2s
H2NCH2CONHBn,
EDCI, i-Pr2NEt, 56%
H2NCH2CONHBn,
EDCI, i-Pr2NEt, 34%
H O
Ar * N N CONHBn
H
t-BuS
40.3s
H O
Ar N N CONHBn
H
t-BuS
40.3f

Scheme 40

from racemic alanine was prepared as summarized in Scheme 40. Again a more polar (**40.3s**) and a less polar (**40.3f**) isomer were obtained, differing in stereochemistry at the starred atom.

It should be noted that in the above sequence the nitrogen was not protected as a carbamate; evidently, the bulky benzylic substituent served as a protecting group. However, if the reactions were to be repeated with optically active alanine ethyl ester, carbamate protection would probably be necessary in order to prevent epimerization.

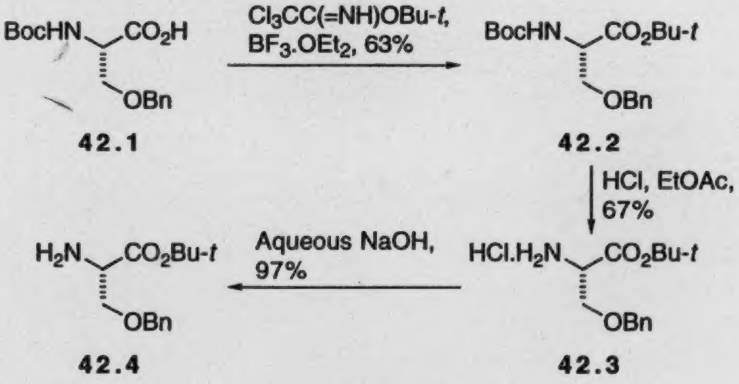
The same series of reactions used with the less polar isomer **39.1f** was carried out with the more polar isomer


Ar
OH
SBu-t
24.1 Ar = 4-MeOC6H4
Me3SiBr,
H2N CO2Bu-t
38.4
H
Ar N CO2Bu-t
t-BuS
39.1f Less polar (31%)
39.1s More polar (30%)
TFA,
anisole,
100%
H
Ar N CO2H
t-BuS
41.1s
TrocCl,
NaOH,
73%
CCl3
O O
Ar N CO2H
t-BuS
41.2s
H2NCH2CONHBn,
HBTU, Et3N, 89%
CCl3
O O O
Ar N N CONHBn
H
t-BuS
41.3s
Cd, DMF,
AcOH, 87%
H O
Ar N N CONHBn
H
t-BuS
41.4s
Hg(OAc)2,
CF3CO2H,
anisole; then
H2S; air, 73%
H O
Ar * N N CONHBn
H
S
2
41.5s

Scheme 41

39.1s to give a final product (**41.5s**) that should differ from **39.6f** only in the stereochemistry at the starred atom.

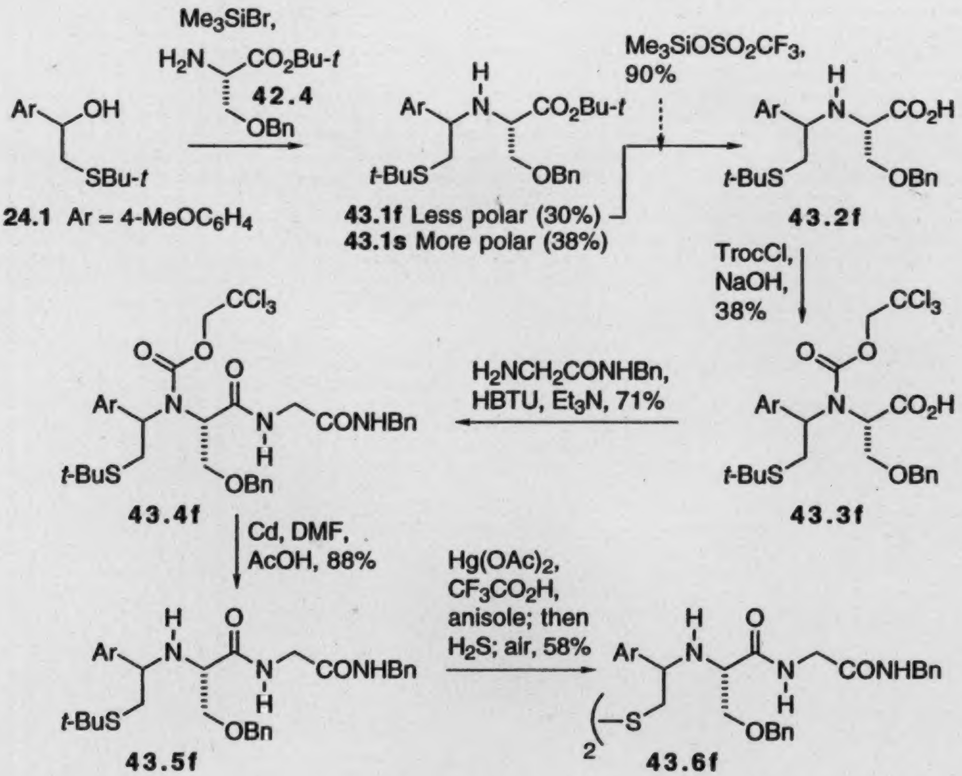
Finally, we decided to study an example of an amino acid with a functionalized side chain, and we chose L-serine as a suitable representative of this class. The required starting material was *O*-benzyl L-serine *t*-butyl ester (**42.4**). Although this is a known compound,[40] several of the literature methods we examined for making it caused extensive epimerization in the step in which the acid was converted[42] into its *t*-butyl ester. We eventually found that the route shown in Scheme 42 is satisfactory.


BocHN
CO2H
OBn
42.1
Cl3CC(=NH)OBu-t,
BF3.OEt2, 63%
BocHN
CO2Bu-t
OBn
42.2
HCl, EtOAc,
67%
HCl.H2N
CO2Bu-t
OBn
42.3
Aqueous NaOH,
97%
H2N
CO2Bu-t
OBn
42.4

Scheme 42

N-Boc *O*-benzyl L-serine[43] could be converted into its *t*-butyl ester **42.2** by the action of *t*-butyl trichloroacetimidate[41] Removal of the Boc group with HCl in EtOAc took place without disturbing the ester, and the free amine (**42.4**) was obtained from the hydrochloride salt by treatment with NaOH. However, the HCl treatment should not be extended beyond 14 h; material obtained at that time (30%)

has an ee of 97%, as judged by ^{19}F NMR measurements on the derived Mosher amide. An additional crop obtained after 36 h (23%) had an ee of 57%. Of course, we used the higher quality batch for subsequent experiments.

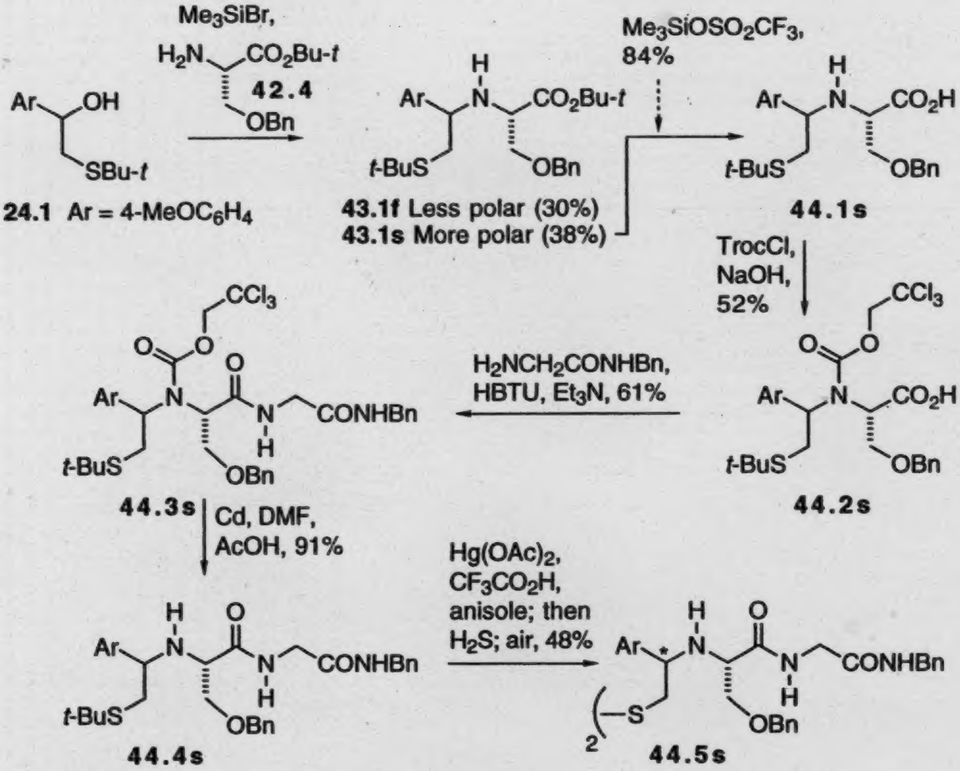


Me3SiBr,
H2N CO2Bu-t
42.4
OBn
Ar
OH
SBu-t
24.1 Ar = 4-MeOC6H4
H
N
CO2Bu-t
t-BuS
OBn
43.1f Less polar (30%)
43.1s More polar (38%)
Me3SiOSO2CF3,
90%
CO2H
43.2f
TrocCl,
NaOH,
38%
CCl3
O
43.3f
H2NCH2CONHBn,
HBTU, Et3N, 71%
CONHBn
43.4f
Cd, DMF,
AcOH, 88%
43.5f
Hg(OAc)2,
CF3CO2H,
anisole; then
H2S; air, 58%
S
2
43.6f

Scheme 43

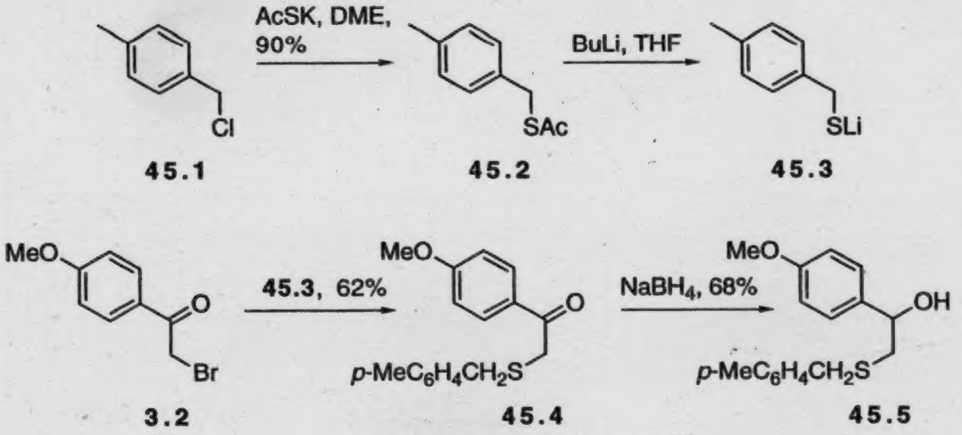
The serine derivative **42.4** was subjected to the series of reactions shown in Scheme 43 and Scheme 44; the reactions are identical to those used for L-alanine, except for the fact that the *t*-butyl ester unit was deprotected with $Me_3SiOSO_2CF_3$, since CF_3CO_2H caused cleavage of the benzylic C-N bond. We have not submitted any of the compounds in this serine series for examination by chiral HPLC.

In order to show additional generality of our method we decided to make compound **46.5**, which has the same sulfur protecting group that was used by Kent[23] and by Dawson[44] in their bromide displacement method.[23] *p*-Methyl benzyl chloride (**45.1**) was converted into thioacetate **45.2**, and treatment with BuLi gave the thiolate **45.3**. This was alkylated with bromide **3.2**, and, finally, reduction gave alcohol **45.5**.


Me3SiBr,
H2N CO2Bu-t
42.4
OBn
Ar OH
SBu-t
24.1 Ar = 4-MeOC6H4
Ar N CO2Bu-t
t-BuS OBn
43.1f Less polar (30%)
43.1s More polar (38%)
Me3SiOSO2CF3, 84%
Ar N CO2H
t-BuS OBn
44.1s
TrocCl, NaOH, 52%
CCl3
44.2s
H2NCH2CONHBn, HBTU, Et3N, 61%
CONHBn
44.3s
Cd, DMF, AcOH, 91%
44.4s
Hg(OAc)2, CF3CO2H, anisole; then H2S; air, 48%
44.5s

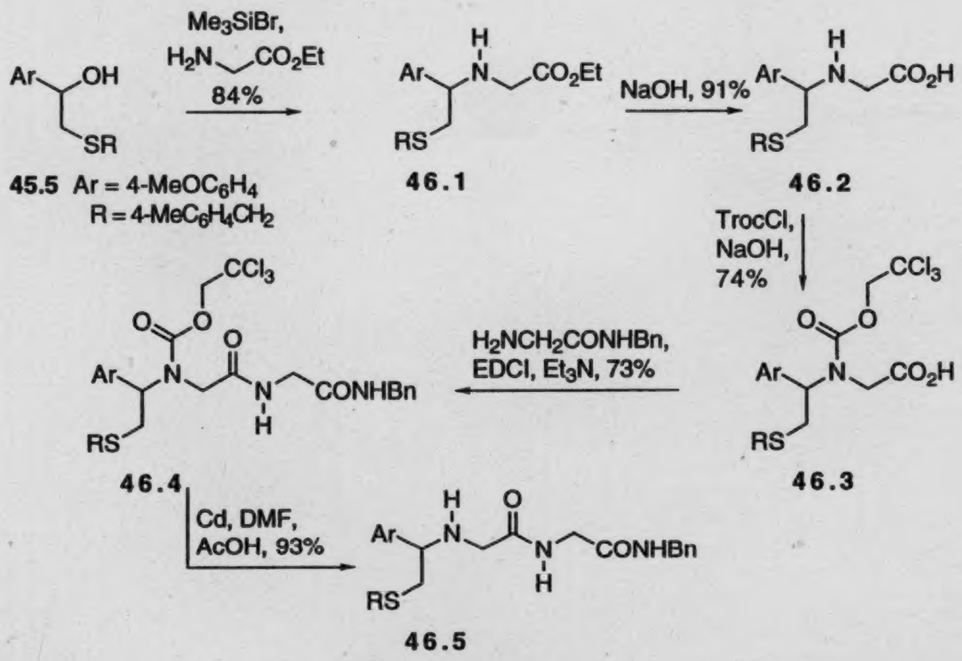
Scheme 44

The alcohol **45.5** was treated with $H_2NCH_2CO_2Et$ and Me_3SiBr, according to our general procedure, and the product **46.1** was hydrolyzed and protected on nitrogen with TrocCl.


AcSK, DME, 90%
BuLi, THF
Cl
SAc
SLi
45.1
45.2
45.3
MeO
O
Br
45.3, 62%
NaBH4, 68%
p-MeC6H4CH2S
OH
3.2
45.4
45.5

Scheme 45

Coupling in the usual way with $H_2NCH_2CONHBn$ and removal of the Troc group gave **46.5**. The protecting group from compounds of this type has been removed with HF.[23] Our procedure clearly


Me3SiBr, H2N CO2Et 84%
Ar
OH
SR
45.5 Ar = 4-MeOC6H4
R = 4-MeC6H4CH2
H
N
CO2Et
RS
46.1
NaOH, 91%
CO2H
46.2
TrocCl, NaOH, 74%
CCl3
O
46.3
H2NCH2CONHBn, EDCI, Et3N, 73%
CONHBn
46.4
Cd, DMF, AcOH, 93%
46.5

Scheme 46

tolerates at least minor changes in the sulfur protecting group.

Conclusion

Our method for making derivatized amino acids of type **1.1** provides an alternative to the bromide displacement route reported by Kent,[23] and has been shown to work with an amino acid having a functionalized side chain, although at the time of writing the stereochemical purity of several compounds has not yet been established. The availability of the derivatized amino acids should be helpful in studies on conformational or steric factors in the derivatizing unit that facilitate or hinder ligation and acyl transfer for different amino acids.

Further studies should include attempts to carry out ligation and acyl transfer with short peptide thioesters, using compounds such as **36.4**, **39.6f**, **41.5s**, **43.6f**, and **44.5s**. Other amino acids should be similarly derivatized and examined in the ligation-acyl transfer sequence. Such studies would identify those amino acids at the ligation site for which the sequence works properly, and those for which acyl transfer is too slow to be useful.

EXPERIMENTAL SECTION

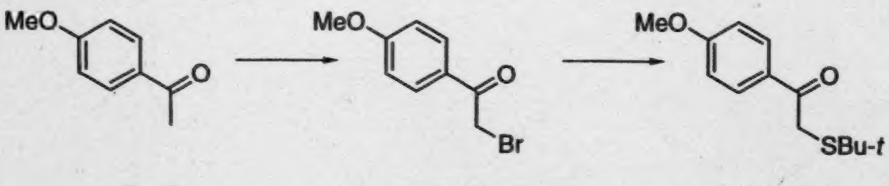
Note: Some of the compounds in this section were not fully characterized as the work was highly exploratory; only those that appeared to lead in the desired direction were fully characterized.

General procedures

Unless stated to the contrary, the procedures described in the Experimental Section of Chapter 1 of this thesis were followed. Optical rotations were measured at 20 °C with a Perkin Elmer 241 Polarimeter, using a sodium lamp.

Compound number **XXf** stands for compound **XX**, faster moving isomer on tlc plates; **XXs** stand for slower moving isomer. The f or s designation in all experiments will indicate whether a particular compound originates from the chromatographically faster- or slower-running series, as determined from the earliest point at which separation of isomers was possible.

2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethanone (3.3).

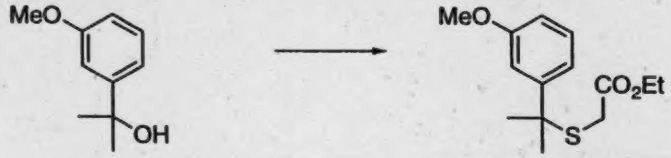


3.1 **3.2** **3.3**

Br_2 (300 µL, 5.77 mmol) was added dropwise over 10 min to a stirred and warmed (40 °C) solution of 4-methoxyacetophenone (**3.1**) (867 mg, 5.77 mmol) in bench $CHCl_3$ (10 mL). At the end of the addition the mixture was diluted with Et_2O (100 mL), washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$) and evaporated to give bromide **3.2** (1.24 g, 93%) as a white solid, which was used for the next step without purification.

A solution of the above bromide (8.82 g, 38.5 mmol) in dry THF (40 mL) was added dropwise over 10 minutes to a stirred and cooled (0 °C) solution of *t*-BuSLi [made by slow addition of *n*-BuLi (2.5 M in hexanes, 17.7 mL, 44.3 mmol) to a stirred and cooled (0 °C) solution of *t*-BuSH (5.21 mL, 46.2 mmol)] in dry THF (100 mL), the solution being stirred for 2.5 h before use]. When addition was complete the cold bath was removed and stirring was continued overnight. The mixture was diluted with Et_2O (200 mL), washed thoroughly with water (3 x 100 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel, using 1:6 EtOAc-hexanes, gave **3.3** (9.05 g, 98%) as a colorless oil: FTIR ($CDCl_3$, cast) 1670 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.34 (s, 9 H), 3.81 (s, 2 H), 3.85 (s, 3 H), 6.88-6.90 (m, 2 H), 7.89-7.91 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 30.7 (q′), 35.5 (t′), 43.6 (s′), 55.4 (q′), 113.8 (d′), 128.7 (s′), 131.1 (d′), 163.6 (s′), 194.9 (s′); exact mass *m/z* calcd for $C_{13}H_{18}O_2S$ 238.1027, found 238.1027.

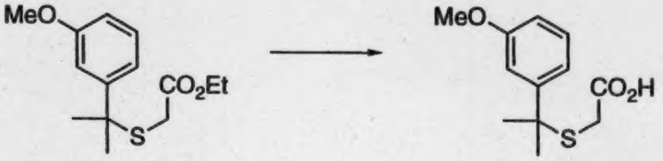
[1-(3-Methoxyphenyl)-1-methylethylsulfanyl]acetic Acid Ethyl Ester (17.5).



17.3 **17.5**

$BF_3.OEt_2$ (42 μL, 0.33 mmol) was added to a stirred and cooled (-78 °C) solution of alcohol **17.3** (50 mg, 0.30 mmol) in dry CH_2Cl_2 (1.5 mL). After 20 min, neat EtO_2CCH_2SH (37 μL, 0.33 mmol) was injected in one portion and the cold bath removed. Stirring was continued for 40 min, by which time all the starting materials were consumed (tlc control, silica, 1:3 EtOAc-hexane). The mixture was diluted with Et_2O (15 mL), washed with water (2 x 5 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 1:6 EtOAc-hexanes, gave **17.5** (75.8 mg, 93%) as a colorless oil: FTIR ($CDCl_3$ cast) 1734 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.17 (t, J = 7.1 Hz, 3 H), 1.68 (s, 6 H), 2.95 (s, 2 H), 3.78 (s, 3 H), 4.02 (q, J = 7.2 Hz, 2 H), 6.71-6.75 (m, 1 H), 7.05-7.09 (m, 2 H), 7.18-7.24 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.0 (q′), 30.1 (two overlapping q′), 32.6 (t′), 48.5 (s′), 55.2 (q′), 61.2 (t′), 111.7 (d′), 112.9 (d′), 118.9 (d′), 129.0 (d′), 147.1 (s′), 159.3 (s′), 170.4 (s′); exact mass m/z calcd for $C_{14}H_{20}O_3S$ 268.1133, found 268.1132.

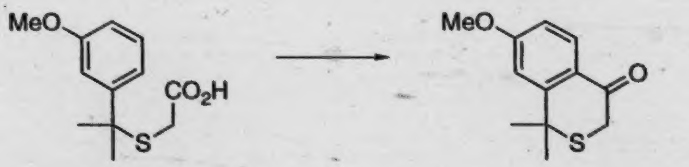
[1-(3-Methoxyphenyl)-1-methylethylsulfanyl]acetic Acid (17.6).



17.5 **17.6**

LiOH.H_2O (3.71 g, 88.3 mmol) was added to a stirred solution of ester **17.5** (2.378 g, 8.83 mmol) in 1:1 water-THF (20 mL). After 5 h the mixture was washed with Et_2O (2 x 20 mL) and the aqueous layer was acidified with concentrated hydrochloric acid. The resulting suspension was extracted with Et_2O (3 x 20 mL) and the combined extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 2:98 MeOH-CH_2Cl_2, gave **17.6** (1.19 g, 90%) as a colorless oil: FTIR ($CDCl_3$, cast) 3500-2600 (br), 1708 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.70 (s, 6 H), 2.99 (s, 2 H), 3.78 (s, 3 H), 6.70-6.73 (m, 1 H), 7.04-7.07 (m, 2 H), 7.19-7.21 (m, 1 H), 11.40 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 29.9 (two overlapping q'), 32.3 (t'), 48.7 (s'), 55.1 (q'), 111.8 (d'), 112.9 (d'), 118.9 (d'), 129.0 (d'), 146.6 (s'), 159.2 (s'), 176.8 (s'); exact mass *m/z* calcd for $C_{12}H_{16}O_3S$ 240.0820, found 240.0817.

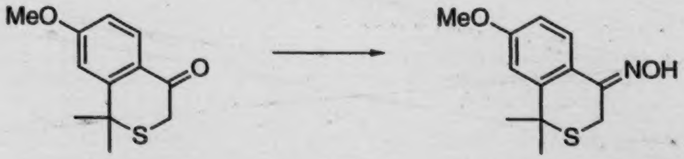
7-Methoxy-1,1-dimethylisothiochroman-4-one (17.7).



17.6 **17.7**

$(CF_3CO)_2O$ (3.2 mL) was added to a stirred and cooled (0 °C) solution of acid **17.6** (389 mg, 1.62 mmol) in CF_3CO_2H (8.0 mL). The cold bath was removed and the brown mixture was stirred for 2 h. The solvent was evaporated and the residue was dissolved in Et_2O (100 mL) and washed with saturated aqueous $NaHCO_3$ (4 x 40 mL). The green ethereal layer was dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 1:6 EtOAc-hexanes, gave **17.7** (336 mg, 93%) as a pale yellow oil: FTIR ($CDCl_3$ cast) 1673 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.71 (s, 6 H), 3.58 (s, 2 H), 3.84 (s, 3 H), 6.72-6.73 (m, 1 H), 6.77-6.79 (m, 1 H), 7.98-8.01 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 30.5 (q'), 34.1 (t'), 41.4 (q'), 55.5 (q'), 110.2 (d'), 111.5 (d'), 124.3 (s'), 131.9 (d'), 152.5 (s'), 163.2 (s'), 190.2 (s'); exact mass *m/z* calcd for $C_{12}H_{14}O_2S$ 222.0715, found 222.0715.

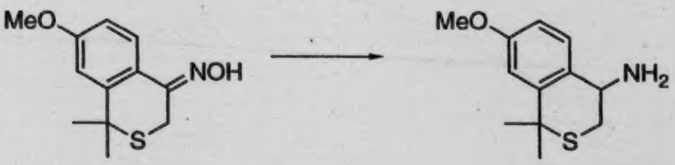
7-Methoxy-1,1-dimethylisothiochroman-4-one Oxime (19.1).



17.7 **19.1**

Ketone **17.7** (131 mg, 0.590 mmol), $NH_2OH.HCl$ (246 mg, 3.54 mmol) and $AcONa.3H_2O$ (480 mg, 3.53 mmol) were dissolved in 1:1 water-EtOH (4 mL) and the mixture was warmed at 40 °C overnight. The solvent was evaporated and the residue was dissolved in Et_2O (30 mL), washed with water (2 x 15 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 25 cm), using 1:6 and then 1:3 EtOAc-hexanes, gave **19.1** (140 mg, 100%) as a white solid: FTIR ($CDCl_3$ cast) 3600-3100 (br), 1603 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.66 (s, 6 H), 3.82 (s, 3 H), 3.96 (s, 2 H), 6.77 (dd, J = 8.7, 2.6 Hz, 1 H), 6.85 (d, J = 2.6 Hz, 1 H), 7.75 (d, J = 8.7 Hz, 1 H), 8.10-8.80 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.7 (t′), 29.1 (two overlapping q′), 41.1 (s′); 54.9 (q′), 108.8 (d′), 111.0 (d′), 121.8 (s′), 127.8 (d′), 147.6 (s′), 151.7 (s′), 160.0 (s′); exact mass m/z calcd for $C_{12}H_{15}NO_2S$ 237.0823, found 237.0821.

7-Methoxy-1,1-dimethylisothiochroman-4-ylamine (19.2).

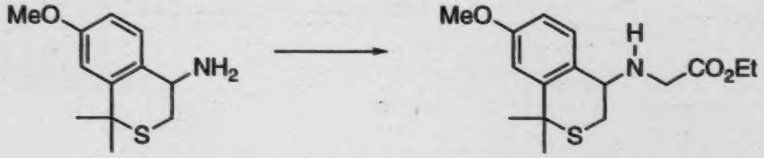


19.1 **19.2**

$NaBH_4$ (768 mg, 19.9 mmol) was added in one portion to a stirred and cooled (0 °C) solution of oxime **19.1** (1.19 g, 4.97 mmol) in dry DME (15 mL) (N_2 atmosphere). After the evolution of gas had subsided, freshly distilled (at 1 atm) $TiCl_4$ (1.09 mL, 9.94 mmol) was added dropwise at 0 °C. The cold bath was left in place but was not recharged, and stirring was continued for 84 h. The resulting blue suspension was diluted with Et_2O (40 mL), cooled (0 °C) and stirred vigorously with concentrated NH_3 in water (40 mL) for 2 h. More Et_2O (200 mL) was added, and the resulting mixture was extracted with 1 N hydrochloric acid (5 x 15 mL). The acid layer was made alkaline (pH 11) with solid NaOH and extracted with Et_2O (3 x 50 mL). The combined extracts were dried ($MgSO_4$) and evaporated to give **19.2** (880 mg, 79%) as a colorless liquid: FTIR ($CDCl_3$ cast) 3300 cm^{-1} (doublet); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.63 (s, 3 H), 1.65 (s, 3 H), 1.94 (br s, 2 H), 2.70 (dd, J_{AM} = 13.8 Hz, J_{AX} = 4.0 Hz, 1 H); 3.27 (dd, J_{AM} = 13.8 Hz, J_{MX} = 3.3 Hz, 1 H), 3.77 (s, 3 H), 3.99 (t, J_{AX} = J_{MX} = 3.6, 1 H), 6.73-6.81 (m, 2 H), 7.20 (d, J =

8.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.3 (q′), 33.1 (t′), 33.7 (q′), 41.7 (s′), 48.9 (d′), 55.2 (q′), 111.2 (d′), 112.0 (d′), 131.5 (d′), 131.8 (s′), 144.2 (s′), 158.4 (s′); exact mass *m/z* calcd for $C_{12}H_{17}NOS$ 223.1031, found 223.1031.

(7-Methoxy-1,1-dimethylisothiochroman-4-ylamino)-acetic Acid Ethyl Ester (20.1).

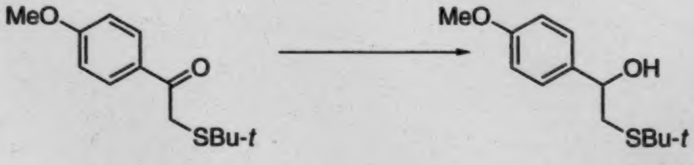


19.2 **20.1**

$BrCH_2CO_2Et$ (484 µL, 4.34 mmol) was added dropwise to a stirred mixture of amine **19.2** (880 mg, 3.95 mmol) and Et_3N (1.10 mL, 7.89 mmol) in dry DME (20 mL). After 36 h the suspension was diluted with Et_20 (50 mL), washed with brine (30 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (3 x 15 cm), using 1:25:75 MeOH-EtOAc-hexanes, gave **20.1** (666 mg, 54%) as a colorless oil: FTIR ($CDCl_3$ cast) 1737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.24 (t, J = 7.2, Hz, 3 H), 1.62 (s, 3 H), 1.64 (s, 3 H), 2.30 (br s, 1 H), 2.81 (dd, J_{AM} = 14.0 Hz, J_{AX} = 4.0 Hz, 1 H), 3.10 (dd, J_{AM} = 14.0 Hz, J_{MX} = 3.0 Hz, 1 H), 3.44 (ABq, $\Delta\nu_{AB}$ = 15.7 Hz, J_{AB} = 17.2 Hz, 2 H), 3.76 (s, 3 H), 3.79 (t, J_{AX} = J_{MX} = 3.4 Hz, 1 H), 4.15 (q, J = 7.1 Hz, 2 H), 6.73 (dd, J = 8.6, 2.7 Hz, 1 H), 6.80 (d, J = 2.6 Hz, 1 H), 7.27 (d, J =

8.5 Hz, 1 H), ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.2 (q′), 29.0 (t′), 31.4 (q′), 34.0 (q′), 41.6 (s′), 48.2 (t′), 54.1 (d′), 55.2 (q′), 60.8 (t′), 111.5 (two overlapping d′), 128.4 (s′), 132.3 (d′), 145.0 (s′), 158.6 (s′), 172.4 (s′); exact mass *m/z* calcd for $C_{16}H_{23}NO_3S$ 309.1399, found 309.1407.

2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethanol (24.1).

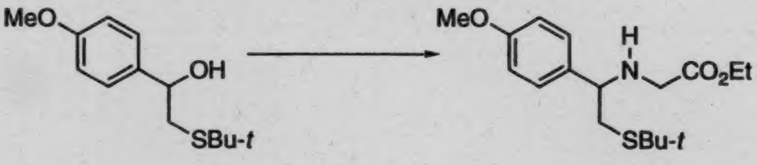


3.3 **24.1**

NaBH$_4$ (400 mg, 10.6 mmol) was added in four approximately equal portions at 15-min intervals to a stirred and cooled (0 °C) solution of ketone **3.3** (826 mg, 3.47 mmol) in dry MeOH (15 mL), and stirring was continued for 30 min at 0 °C. The solvent was evaporated and the residue was dissolved in a 1:1 mixture of water and EtOAc (80 mL) and stirred vigorously for 1 h. The organic layer was separated, and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layer and extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (3 x 25 cm), using 1:3 EtOAc-hexanes, gave **24.1** (760 mg, 92%) as a colorless oil: FTIR ($CDCl_3$ cast) unexceptional; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.33 (s, 9H), 2.72-2.77 (m containing a

broad singlet, 2 H in all), 2.88-2.92 (m, 1 H), 3.78 (s, 3 H), 4.67 (dd, J = 9.4, 3.8 Hz, 1 H), 6.84-6.85 (m, 2 H), 7.25-7.27 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 31.2 (q′), 38.7 (t′), 42.7 (s′), 55.3 (q′), 72.1 (d′), 113.8 (d′), 126.9 (d′), 134.9 (s′), 159.1 (s′); exact mass m/z calcd for $C_{13}H_{20}O_2S$ 240.1184, found 240.1187.

[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethylamino]acetic Acid Ethyl Ester (24.2).

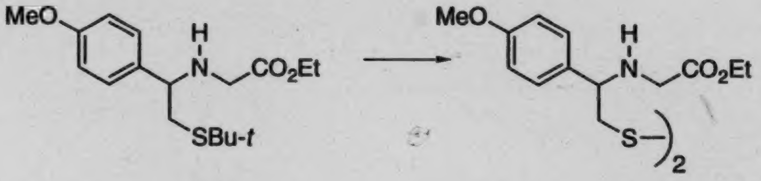


24.1 **24.2**

Me_3SiBr (63 μL, 0.48 mmol) was added dropwise to a stirred and cooled (0 °C) solution of alcohol **24.1** (114 mg, 0.475 mmol) in dry CH_2Cl_2 (2.0 mL). After 30 min, freshly distilled (distilled under water pump vacuum) $H_2NCH_2CO_2Et$ (98 mg, 0.95 mmol) was added in one portion. The cold bath was removed and stirring was continued for 1 h. Evaporation of the solvent, and flash chromatography of the residue over silica gel (2 x 15 cm), using 1:6 EtOAc-hexanes, gave **24.2** (105 mg, 67%) as a colorless oil: FTIR ($CDCl_3$ cast) 1738 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.22 (t, J = 7.1 Hz, 3 H), 1.31 (s, 9 H), 2.60 (br s, 1 H), 2.73 (dd, J_{AB} = 12.3 Hz, J_{AX} = 9.1 Hz, 1 H), 2.81 (dd, J_{AB} = 12.3 Hz, J_{BX} = 4.7 Hz, 1 H), 3.21 (ABq,

$\Delta\nu_{AB}$ = 48.5 Hz, J_{AB} = 17 Hz, 2 H), 3.73 (dd, J_{AX} = 9.1 Hz, J_{BX} = 4.6 Hz, 1 H), 3.78 (s, 3 H), 4.14 (q, J = 7.1 Hz, 2 H), 6.86 (d, J = 8.7 Hz, 2 H), 7.24 (d, J = 8.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.3 (q′), 31.1 (q′), 37.0 (t′), 42.5 (s′), 48.8 (t′), 55.3 (q′), 60.7 (t′), 61.6 (d′), 113.9 (d′), 128.2 (d′), 134.2 (s′), 159.0 (s′), 172.1 (s′); exact mass m/z calcd. for $C_{17}H_{27}NNaO_3S$ (M + Na) 348.1609, found 348.1610.

[2-[2-(Ethoxycarbonylmethylamino)-2-(4-methoxyphenyl)ethyldisulfanyl]-1-(4-methoxyphenyl)ethylamino]acetic Acid Ethyl Ester (25.1).

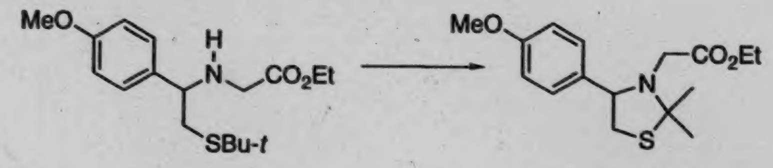


24.2 **25.1**

CF_3CO_2H (0.5 mL) was added to thioether **24.2** (54.7 mg, 0.168 mmol) contained in a flask immersed in an ice bath. The mixture was stirred and $Hg(OAc)_2$ (54.0 mg, 0.168 mmol) was added in one portion. Stirring was continued for 15 min at 0 °C. The solvent was evaporated and the residue was dissolved in MeCN (15 mL) and H_2S gas was bubbled through the solution for 2 min. The resulting black suspension was filtered through a tightly packed Celite column (2 x 4 cm) and the solid was washed with several portions of MeCN.

Evaporation of the combined filtrate and washings, and flash chromatography of the residue over silica gel (2 x 18 cm), using 4:100 MeOH-CH_2Cl_2, gave disulfide **25.1** (31.9 mg, 70%) as a glassy liquid: FTIR ($CDCl_3$ cast) 1735 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.23 (t, J = 7.1 Hz, 3 H), 2.70 (dd, J_{AB} = 13.5 Hz, J_{AX} = 7.9 Hz, 1 H), 2.83 (dd, J_{AB} = 13.4 Hz, J_{BX} = 5.5 Hz, 1 H), 3.25 (ABq, $\Delta\nu_{AB}$ = 44.9 Hz, J_{AB} = 17.4 Hz, 2 H), 3.70 (dd, J_{AX} = 7.9 Hz, J_{BX} = 5.5 Hz, 1 H), 3.79 (s, 3 H), 4.14 (q, J = 7.4 Hz, 2 H), 6.85-6.87 (m, 2 H), 7.20-7.24 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (q′), 31.9 (t′), 48.3 (t′), 55.2 (q′), 60.8 (t′), 64.0 (d′), 114.1 (d′), 128.6 (d′), 132.5 (s′), 159.3 (s′), 171.9 (s′); exact mass *m/z* calcd. for $C_{26}H_{36}N_2NaO_6S_2$ (M + Na) 559.1912, found 559.1912.

[4-(4-Methoxyphenyl)-2,2-dimethylthiazolidin-3-yl]acetic Acid Ethyl Ester (25.2).

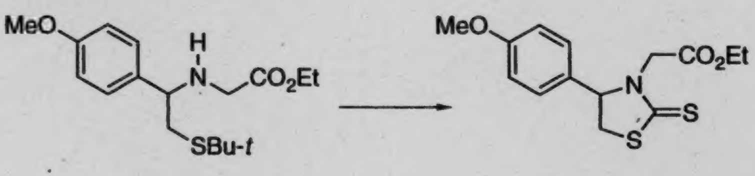


24.2 **25.2**

CF_3CO_2H (3 mL) was added to thioether **24.2** (466 mg, 1.43 mmol) contained in a flask immersed in an ice-bath. The mixture was stirred and PhOMe (0.23 mL), followed by $Hg(OAc)_2$ (458 mg, 1.43 mmol) were added. Stirring was continued for 25 min and the solvent was evaporated. The residue was dissolved in MeCN (15 mL) and H_2S gas was bubbled through the solution for 2 min. The resulting black suspension was filtered through a tightly packed Celite column (2 x 4 cm) and the solid was washed with several portions of MeCN. Evaporation of the combined filtrate and washings gave crude disulfide **25.1**, which was used directly.

A mixture of disulfide **25.1**, *p*-TsOH.H_2O (10 mg) and Bu_3P (1.08 mL, 8.6 mmol) in 2,2-dimethoxypropane (10 mL) was refluxed for 2 h (N_2 atmosphere) and then left overnight at room temperature. Evaporation of the solvent and flash chromatography of the residue over silica gel (2 x 20 cm), using 1:7, 1:6, 1:5 and then 1:3 EtOAc-hexanes, gave **25.2** (288 mg, 65%) as a colorless oil: FTIR ($CDCl_3$ cast) 1742 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.14 (t, J = 7.1 Hz, 3 H), 1.49 (s, 3 H), 1.58 (s, 3 H), 2.93 (dd, J_{AB} = 10.3 Hz, J_{AX} = 9.6 Hz, 1 H), 3.06 (dd, J_{AB} = 10.4 Hz, J_{BX} = 6.1 Hz, 1 H), 3.18 (s, 2 H), 3.76 (s, 3 H), 3.92-3.98 (m, 2 H), 4.49 (dd, J_{AX} = 9.5 Hz, J_{BX} = 5.9 Hz, 1 H), 6.80-6.82 (m, 2 H), 7.27-7.30 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2 (q′), 27.5 (q′), 30.9 (q′), 37.4 (t′), 47.0 (t′), 55.3 (q′), 60.3 (t′), 68.7 (d′), 71.6 (s′), 113.8 (d′), 129.2 (d′), 132.7 (s′), 159.2 (s′), 172.1 (s′); exact mass *m/z* calcd for $C_{16}H_{23}NNaO_3S$ (M + Na) 332.1296, found 332.1298.

[4-(Methoxyphenyl)-2-thioxothiazolidin-3-yl]acetic Acid Ethyl Ester (27.1).



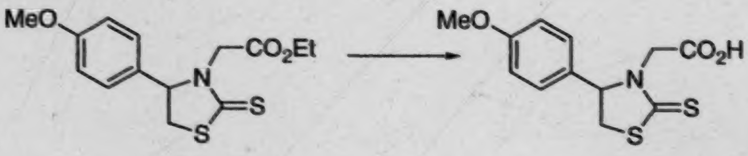
24.2 **27.1**

CF_3CO_2H (1 mL) was added to thioether **24.2** (84.3 mg, 0.268 mmol) contained in a flask immersed in an ice-bath. The mixture was stirred and PhOMe (0.04 mL), followed by $Hg(OAc)_2$ (85.4 mg, 0.268 mmol) were added. Stirring was continued for 25 min and the solvent was evaporated. The residue was dissolved in MeCN (15 mL) and H_2S gas was bubbled through the solution for 2 min. The resulting black suspension was filtered through a tightly packed Celite column (2 x 4 cm) and the solid was washed with several portions of MeCN. Evaporation of the combined filtrate and washings gave crude disulfide **25.1**, which was used directly.

Bu_3P (67 μL, 0.27 mmol) and 1,1′-thiocarbonyldiimidazole (106 mg, 0.53 mmol) were added to a stirred solution of the above disulfide **25.1** in dry PhH (6 mL), and the mixture was stirred overnight (N_2 atmosphere). Evaporation of the solvent and flash chromatography of the residue over silica gel (2 x 18 cm), using 1:3 EtOAc-hexanes and then 1:66:33 MeOH-EtOAc-hexanes, gave **27.1** (44.8 mg, 55%) as a yellow solid: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.22 (t, J = 7.2 Hz, 3 H),

3.28 (dd, J_{AB} = 11.2 Hz, J_{AX} = 8.8 Hz, 1 H), 3.53 (d, J = 17.3 Hz, 1 H), 3.63 (dd, J_{AB} = 11.2 Hz, J_{BX} = 8.8 Hz, 1 H), 3.80 (s, 3 H), 4.08-4.19 (m, 2 H), 4.97 (d, J = 17.3 Hz, 1 H), 5.36 (t, J_{AX} = J_{BX} = 8.5 Hz, 1 H), 6.90-6.93 (m, 2 H), 7.19-7.23 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2 (q′), 35.9 (t′), 48.0 (t′), 55.5 (q′), 61.6 (t′), 71.6 (d′), 114.8 (d′), 128.6 (d′), 128.9 (s′), 160.4 (s′), 167.0 (s′).

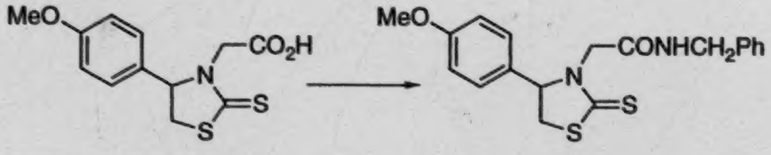
[4-(Methoxyphenyl)-2-thioxothiazolidin-3-yl]-acetic Acid (27.2).



27.1 **27.2**

A mixture of ester **27.1** (44.8 mg, 0.149 mmol) and $LiOH.H_2O$ (125 mg, 2.98 mmol) in 2:1 dioxane-water (1.5 mL) was stirred vigorously at room temperature for 15 min. The mixture was poured into an ice-water mixture (30 mL) and the solution was adjusted to pH 2 with concentrated hydrochloric acid. The resulting suspension was extracted with Et_2O (3 x 30 mL), and the combined extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (1.2 x 15 cm), using 1:49 AcOH-CH_2Cl_2, gave **27.2** (26.0 mg, 64%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.31 (dd, J_{AB} = 11.2 Hz, J_{AX} = 9.0 Hz, 1 H), 3.60-3.70 [overlapping signals including d at δ 3.62 (J = 17.6 Hz, 1 H), a dd at δ 3.64 (J_{AB} = 11.2 Hz, J_{BX} = 8.0 Hz, 1 H)], 3.82 (s, 3 H), 4.88 (d, J = 17.7 Hz, 1 H), 5.33 (t, J_{AX} = J_{BX} = 8.6 Hz, 1 H), 6.94 (d, J = 8.7 Hz, 2 H), 7.24 (d, J = 9.0 Hz, 2 H).

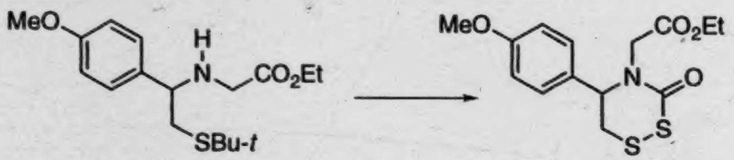
***N*-Benzyl-2-[4-(4-methoxyphenyl)-2-thioxothiazolidin-3-yl]acetamide (27.3).**



27.2 **27.3**

EDCI (18.6 mg, 0.095 mmol) was added to a stirred and cooled (0 °C) mixture of acid **27.2** (26 mg, 0.095 mmol) and $BnNH_2$ (10.4 µL, 0.0952 mmol) in dry CH_2Cl_2 (2 mL). The cold bath was left in place but was not recharged, and stirring was continued overnight. The mixture was diluted with Et_2O (30 mL), washed with water (3 x 10 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel, using 1:24:75 MeOH-EtOAc-hexanes, gave **27.3** (24.3 mg, 70%) as a white solid: FTIR ($CDCl_3$, cast) 3296 (br), 1660, 1610 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.24 (dd, J_{AB} = 11.3 Hz, J_{AX} = 7.4 Hz, 1 H), 3.59 (d, J = 15.2 Hz, 1 H), 3.66 (dd, J_{AB} = 11.3 Hz, J_{BX} = 8.6 Hz, 1 H), 3.78 (s, 3 H), 4.36 (d, J = 5.9 Hz, 2 H), 4.79 (d, J = 15.2 Hz, 1 H), 5.36 (dd, J_{AX} = 7.7 Hz, J_{BX} = 8.2 Hz, 1 H), 6.42 (br s, 1 H), 6.86-6.88 (m, 2 H), 7.18-7.29 (m, 7 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 35.6 (t′), 43.5 (t′), 50.5 (t′), 55.4 (q′), 72.0 (d′), 114.8 (d′), 127.5 (d′), 127.6 (d′), 128.6 (d′), 128.7 (d′), 129.0 (s′), 137.6 (s′), 160.5 (s′), 166.4 (s′), 198.5 (s′).

[5-(4-Methoxyphenyl)-3-oxo-[1,2,4]dithiazinan-4-yl]acetic Acid Ethyl Ester (28.1).

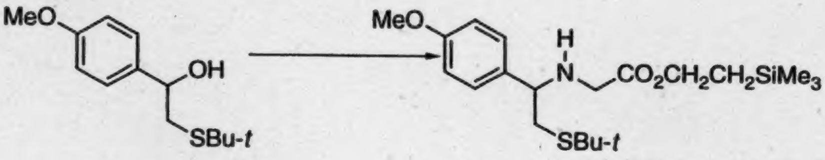


24.2 **28.1**

Chlorocarbonylsulfenyl chloride [ClC(O)SCl] (94 μL, 1.1 mmol) was added dropwise to a stirred and cooled (-5 °C) mixture of thioether **24.2** (266 mg, 1.11 mmol) and Et_3N (155 μL, 1.50 mmol) in dry CH_2C_{12} (10 mL). The cold bath was left in place but was not recharged, and stirring was continued overnight. Evaporation of the solvent, and flash chromatography of the residue over silica gel (2 x 20 cm), using 1:5 and then 1:3 EtOAc-hexanes, gave **28.1** (78.2 mg, 29%) as a brown liquid: FTIR ($CDCl_3$ cast) 1743, 1624 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.23 (t, J = 7.2 Hz, 3 H), 3.16 (dd, J_{AB} = 14.2 Hz, J_{AX} = 4.4 Hz, 1 H), 3.29 (d, J = 17.4 Hz, 1 H), 3.66 (dd, J_{AB} = 14.2 Hz, J_{BX} = 4.4 Hz, 1 H), 3.79 (s, 3 H), 4.12-4.17 (m, 2 H), 4.56 (d, J = 17.3, Hz, 1 H), 5.07 (t, J_{AX} = J_{BX} = 4.4 Hz, 1 H), 6.88 (d, J = 8.7 Hz, 2 H), 7.20 (d, J =

8.7 Hz, 2 H); exact mass *m/z* calcd for $C_{14}H_{18}NO_4S_2$ (M + H) 328.0677, found 328.0677.

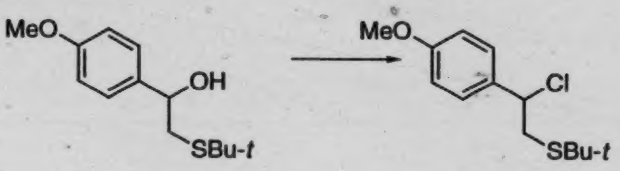
[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethylamino]acetic Acid 2-(Trimethylsilyl)ethyl Ester (30.1).



24.1 **30.1**

Me_3SiBr (158 μL, 1.20 mmol) was added dropwise to a stirred solution of alcohol **24.1** (287 mg, 1.20 mmol) in dry CH_2Cl_2 (5 mL). After 40 min, neat amine **29.3**[32] (418 mg, 2.39 mmol) was injected in one portion, and stirring was continued for 24 h at room temperature. The mixture was poured into Et_2O (50 mL), washed with saturated aqueous $NaHCO_3$ (2 x 15 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 1:10, 1:7 and then 1:5 EtOAc-hexanes, gave **30.1** (235 mg, 49%) as a pale yellow oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 0.0 (s, 9 H), 0.91-0.97 (m, 2 H), 1.30 (s, 9 H), 2.69-2.84 (m, 2 H), 3.19 (ABq, $\Delta\nu_{AB}$ = 37.9 Hz, J_{AB} = 17.3 Hz, 2 H), 3.73 (dd, J = 9.1, 4.9 Hz, 1 H), 3.77 (s, 3 H), 4.13-4.19 (m, 2 H), 6.83-6.86 (m, 2 H), 7.23-7.25 (m, 2 H).

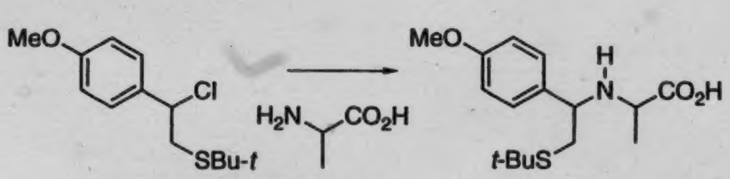
1-(2-*tert*-Butylsulfanyl-1-chloroethyl)-4-methoxybenzene (32.1).



24.1 **32.1**

$SOCl_2$ (347 μL, 4.67 mmol) was added dropwise to a stirred and cooled (0 °C) solution of alcohol **24.1** (1.22 g, 4.67 mmol) in dry CH_2Cl_2 (6 mL). After 2 h the mixture was diluted with Et_2O (80 mL), washed with saturated aqueous $NaHCO_3$ (3 x 40 mL), dried ($MgSO_4$) and evaporated to give the crude chloride **32.1** (1.16 g, 96%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.29 (s, 9 H), 3.21 (d, *J* = 8.0 Hz, 2 H), 3.78 (s, 3 H), 4.93 (t, *J* = 7.6 Hz, 1 H), 6.84-6.88 (m, 2 H), 7.28-7.31 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 30.9 (q′), 37.4 (t′), 43.0 (s′), 55.2 (q′), 62.3 (d′), 114.0 (d′), 128.7 (d′), 132.2 (s′), 159.7 (s′). The chloride was used crude.

2-[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethylamino]propionic Acid (32.2).



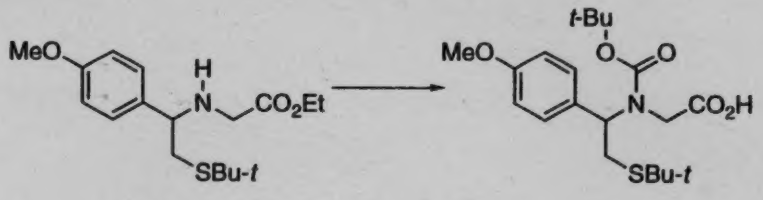
32.1 **32.2**

Note: This experiment was done to show that a chloride could be used and that an unprotected amino acid is suitable for reaction with the aromatic unit.

Me_3SiCl (3 mmol, 381 μL) was added to a stirred suspension of (±)-alanine (267 mg, 3 mmol) in dry CH_2Cl_2 (6 mL) and the mixture was refluxed for 5 h. Et_3N (3 mmol, 420 μL) was then added to the cooled mixture and stirring was continued for 20 min.

A solution of chloride **32.1** (729 mg, 2.82 mmol) in dry CH_2Cl_2 (3 mL) was added to the above mixture, which was then refluxed overnight. The mixture was cooled and poured into water (20 mL). The aqueous layer was adjusted to pH 10 with 5 N NaOH. The alkaline solution was thoroughly washed with Et_2O (3 x 20 mL) and then carefully treated with concentrated hydrochloric acid to ca pH 2. The hydrochloride of **32.2** (324 mg, 31%) separated out as a brown solid consisting of an inseparable mixture of diastereoisomers: ^1H NMR ($CDCl_3$, 400 MHz) δ 1.22-1.30 (overlapping singlets at δ 1.26 and δ 1.27, 9 H in all), 1.50-1.64 [overlapping doublets at δ 1.53 (J = 6.7 Hz) and δ 1.58 (J = 6.7 Hz), 3 H in all], 3.22-3.60 (m, 3 H), 3.68-3.80 (overlapping singlets at δ 3.76 and δ 3.78, 3 H in all), 4.21-4.38 (m, 1 H), 6.84-6.94 [overlapping doublets at δ 6.88 (J = 8.6 Hz) and δ 6.90 (J = 8.7 Hz), 2 H in all], 7.46-7.58 [overlapping doublets at δ 7.49 (J = 7.6 Hz) and δ 7.54 (J = 8.2 Hz), 2 H in all]; ^{13}C NMR ($CDCl_3$, 100 MHz) δ (mixture of diastereoisomers) 16.6 (q′), 30.91 (q′), 30.97 (q′), 31.7 (s′), 32.0 (s′), 43.3 (t′), 45.3 (t′), 54.4 (d′), 55.1 (q′), 60.8 (d′), 62.9 (d′), 114.6 (d′), 124.8 (s′), 125.1 (s′), 130.1 (d′), 130.4 (d′), 160.4 (s′), 160.5 (s′), 172.5 (s′).

[*tert*-Butoxycarbonyl[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethyl]amino]acetic Acid (36.1).



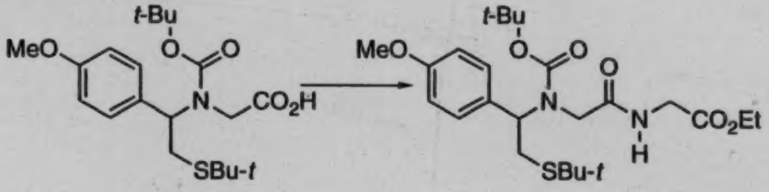
24.2 **36.1**

$LiOH.H_2O$ (154 mg, 3.66 mmol) was added to a stirred solution of ester **24.2** (595 mg, 1.83 mmol) in 1:1 THF-water (3 mL), and the mixture was stirred vigorously for 72 h, diluted with water (15 mL) and thoroughly washed with Et_2O (3 x 10 mL). The aqueous layer was concentrated to ca 3 mL, mixed with dioxane (3 mL) and cooled (0 °C). Boc_2O (483 mg, 2.01 mmol) was added at 0 °C in three approximately equal portions over 1.5 h with vigorous stirring. Stirring was continued at 0 °C for 4 h, during which time a precipitate formed. The mixture was extracted with EtOAc (3 x 25 mL) and the aqueous layer was chilled (0 °C) and its pH was carefully adjusted to 7 with concentrated hydrochloric acid. The aqueous phase was extracted with EtOAc (2 x 10 mL). The combined extracts were dried ($MgSO_4$) and evaporated to give

36.1 (497 mg, 68%) as a thick oil. Attempted chromatography over silica gel or neutral alumina (G III), using 4:96 MeOH-CH_2Cl_2, led to decomposition and loss of **36.1**. Acid **36.1** was used crude for the next step.

For characterization, the cyclohexylammonium salt of acid **36.1** was prepared as follows: Cyclohexylamine (114 μL, 1.20 mmol) was added to a stirred solution of acid **36.1** (199 mg, 0.50 mmol). After 30 min, hexane was added and the resulting precipitate was filtered and recrystallized from EtOAc-hexane, to give the ammonium salt (163 mg, 33%) as a white solid: mp 108-113 °C; FTIR (microscope) 3600-2100 (br), 1744, 1612 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.05-1.48 (overlapping signals containing two singlets at δ 1.30 and δ 1.43, 23 H in all), 1.59 (d, J = 12.5 Hz, 1 H), 1.70 (d, J = 12.0 Hz, 2 H), 1.93 (d, J = 11.0 Hz, 2 H), 2.74-2.84 (m, 1 H), 3.20-3.30 (m, 3 H), 3.46-3.60 (m, 1 H), 3.75 (s, 3 H), 5.15 (br s, 0.6 H), 5.34 (br s, 0.4 H), 6.79 (d, J = 7.6 Hz, 2 H), 7.20 (d, J = 8.3 Hz, 2 H), 7.50-7.80 (br s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (mixture of rotamers) 24.7 (t′), 25.0 (t′), 28.6 (q′), 30.2 (s′), 31.0 (q′), 31.7 (t′), 42.8 (t′), 48.1 (t′), 50.1 (d′), 55.2 (q′), 60.2 (d′), 80.0 (s′), 113.6 (d′), 129.3 (d′), 129.7 (d′), 131.0 (s′), 155.4 (s′), 158.7 (s′), 175.3 (s′); exact mass *m/z* calcd for $C_{26}H_{45}N_2O_5S$ (M + H) 497.3049, found 497.3053.

[[*tert*-Butoxycarbonyl[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethyl]amino]acetylamino]acetic Acid Ethyl Ester (36.2).

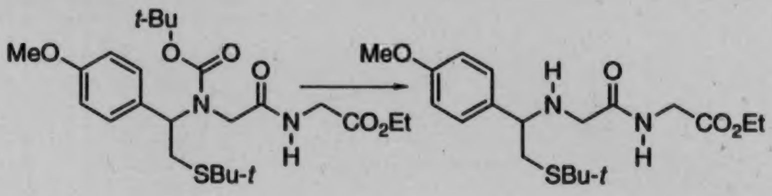


36.1 **36.2**

Et_3N (745 µL, 0.530 mmol) was added to a stirred suspension of ethyl glycine hydrochloride (103 mg, 0.750 mmol) in dry CH_2Cl_2 (3 mL), and the mixture was stirred for 15 min. A solution of acid **36.1** (210 mg, 0.530 mmol) in dry CH_2Cl_2 (2 mL) was added to the resulting solution of $H_2NCH_2CO_2Et$. The stirred mixture was cooled (0 °C) and EDCI (101 mg, 0.530 mmol) was added, followed by DMAP (5 mg). Stirring at 0 °C was continued for 1.5 h, by which time the reaction was complete (tlc control, silica, 4:96 MeOH-CH_2Cl_2). The mixture was poured into Et_2O (40 mL), washed successively with water (2 x 15 mL) and saturated aqueous $NaHCO_3$ (2 x 15 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 4:96 MeOH-CH_2Cl_2, gave **36.2** (163 mg, 64%) as an oil: FTIR ($CDCl_3$ cast) 3324 (br), 1750, 1689 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.25 (t, J = 7.1 Hz, 3 H); 1.32 (s, 9 H), 1.48 (br s, 9 H), 3.19-3.29 (m, 2 H), 3.50-3.62 (m, 2 H), 3.79 (s, 3 H), 3.80-4.00 (m, 2

H), 4.18 (q, J = 7.1 Hz, 2 H), 5.34 (br s, 1 H), 6.82-6.85 (m, 2 H), 7.03 (br t, 1 H), 7.19-7.24 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (q′), 28.3 (q′), 30.1 (t′), 30.9 (q′), 41.1 (t′), 42.6 (t′), 47.8 (s′), 55.2 (overlapping q′ and d′), 61.2 (t′), 81.4 (s′), 114.0 (d′), 128.9 (d′), 130.6 (s′), 159.3 (s′), 169.2 (s′), 170.2 (s′); exact mass m/z calcd for $C_{24}H_{38}N_2NaO_6S$ 505.2348 (M + Na), found 505.2347.

[[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethylamino]acetylamino]acetic Acid Ethyl Ester (36.3).

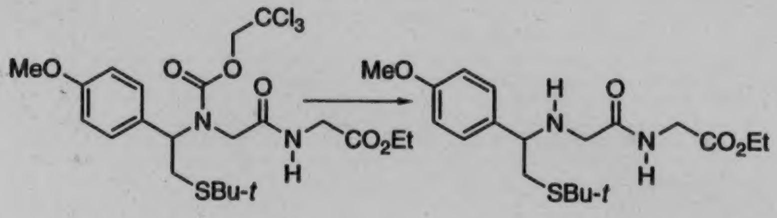


36.2 **36.3**

$Me_3SiOSO_2CF_3$ (18.3 µL, 0.101 mmol) was added dropwise to a stirred and cooled (0 °C) solution of **36.2** (24.2 mg, 0.050 mmol) in dry CH_2Cl_2 (0.5 mL). The mixture was stirred for 2 h at 0 °C, diluted with Et_2O (50 mL) and washed with saturated aqueous $NaHCO_3$ solution (10 mL). The organic layer was dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 9 cm), using CH_2Cl_2 and then 4:96 MeOH-CH_2Cl_2, gave **36.3** (6.9 mg, 36%) as a colorless oil: FTIR ($CDCl_3$ cast) 3307 (br), 1748, 1672, 1609 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.27 (t, J = 7.2, Hz, 3 H); 1.30 (s, 9 H), 1.60

(br s, 1 H), 2.71-2.81 (m, 2 H), 3.17 (ABq, $\Delta\nu_{AB}$ = 38.2 Hz, J_{AB} = 17.1 Hz, 2 H), 3.68 (dd, J = 9.5, 4.4 Hz, 1 H), 3.77 (s, 3 H), 3.98 (dd, J = 5.6, 3.1 Hz, 2 H), 4.19 (q, J = 3.1 Hz, 2 H), 6.81-6.83 (m, 2 H), 7.17-7.19 (m, 2 H), 7.70-7.72 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.3 (q′), 31.1 (q′), 36.5 (t′), 40.9 (t′), 42.7 (s′), 49.9 (t′), 55.3 (q′), 61.4 (t′), 62.4 (d′), 114.1 (d′), 128.0 (d′), 133.6 (s′), 159.2 (s′), 169.6 (s′), 171.6 (s′); exact mass *m/z* calcd for $C_{19}H_{31}N_2O_4S$ (M + H) 383.2004, found 383.2002.

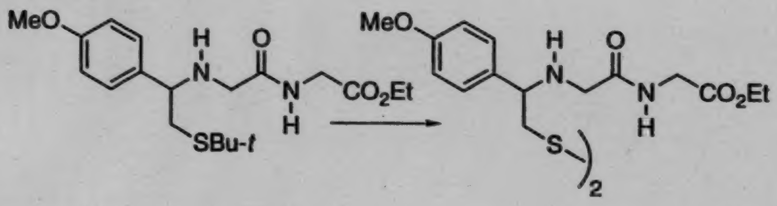
[2-[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)-ethylamino]acetylamino]acetic Acid Ethyl Ester (36.3).



37.3 **36.3**

Cd powder (1.20 g, 10.7 mmol) was added in one portion to a stirred solution of **37.3** (179 mg, 0.322 mmol) in 1:1 DMF-AcOH (8 mL). Stirring was continued for 45 min at room temperature, and the mixture was filtered through a Celite pad (2 x 4 cm), using EtOAc (3 x 20 mL). The combined filtrates and washings were washed with saturated aqueous $NaHCO_3$ (2 x 15 mL) and water (15 mL), dried ($MgSO_4$) and evaporated. The residue was kept under oil pump vacuum to remove residual DMF, and crude amine **36.3** (118 mg, 97%) was obtained as a pale yellow oil, which had the same spectral data as those measured for the product made from the corresponding *N*-Boc starting material (**36.2**).

[2-[2-[2-[[(Ethoxycarbonylmethylcarbamoyl)-methyl]amino]-2-(4-methoxyphenyl)ethyldisulfanyl]-1-(4-methoxyphenyl)ethylamino]acetylamino]acetic Acid Ethyl Ester (36.4).

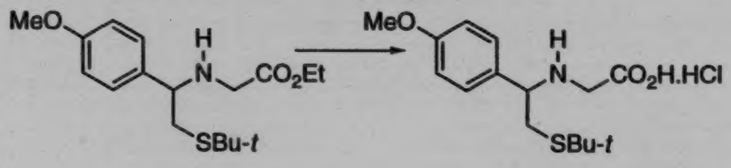


36.3 **36.4**

In this experiment the initial product was not protected from air, and so the disulfide was obtained.

CF_3CO_2H (2 mL) was added to thioether **36.3** (118 mg, 0.311 mmol) contained in a flask immersed in an ice-bath. The mixture was stirred and PhOMe (50 μL), followed by $Hg(OAc)_2$ (99.3 mg, 0.311 mmol) were added. Stirring was continued for 25 min and the solvent was evaporated. The residue was dissolved in MeCN (15 mL) and H_2S gas was bubbled through the solution for 2 min. The resulting black suspension was filtered through a tightly packed Celite column (2 x 4 cm) and the solid was washed with several portions of MeCN. Evaporation of the combined filtrate and washings, and flash chromatography of the residue over silica gel (2 x 18 cm), using 4:100 MeOH-CH_2Cl_2, gave disulfide **36.4** (92.4 mg, 92%) as a colorless oil: FTIR ($CDCl_3$ cast) 1745, 1668 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.26 (t, J = 7.1 Hz, 6 H), 2.74 (dd, J_{AB} = 13.7 Hz, J_{AX} = 8.0 Hz, 2 H), 3.23 (ABq, $\Delta\nu_{AB}$ = 15.4 Hz, J_{AB} = 17.0 Hz, 4 H), 2.85 (dd, J_{AB} = 13.7 Hz, J_{BX} = 5.3 Hz, 2 H), 3.71 (dd, J_{AX} = 7.8 Hz, J_{BX} = 5.4 Hz, 2 H), 3.76 (s, 6 H), 3.96 (dd, J_{AB} = 18.3 Hz, J_{AX} = 5.4 Hz, 2 H), 4.02 (dd, J_{AB} = 18.3 Hz, J_{BX} = 5.6 Hz, 2 H), 4.19 (q, J = 7.1 Hz, 4 H), 6.82-6.85 (m, 4 H), 7.14-7.17 (m, 4 H), 7.65 (br t, J = 4.5 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2 (q′), 31.6 (t′), 41.0 (t′), 49.5 (t′), 55.3 (q′), 61.5 (t′), 64.3 (d′), 114.2 (d′), 128.3 (d′), 131.8 (s′), 159.4 (s′), 169.7 (s′), 170.9 (s′); exact mass *m/z* calcd for $C_{30}H_{43}N_4O_8S$ (M + H) 651.2522, found 651.2524.

[2-tert-Butylsulfanyl-1-(4-methoxyphenyl)ethylamino]acetic Acid Hydrochloride (37.1).

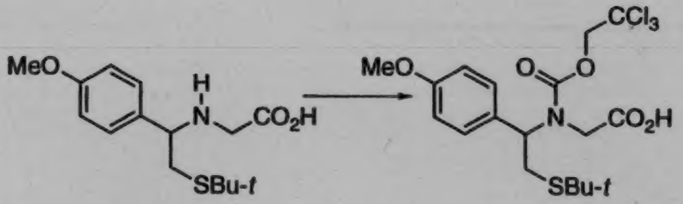


24.2 **37.1**

NaOH (1.29 g, 32.4 mmol) was added to a stirred solution of ester **24.2** (1.034 g, 3.24 mmol) in 1:1 THF-water (28 mL).

After 4 h the mixture was diluted with water (25 mL) and washed with Et_2O (3 x 25 mL). The aqueous layer was cooled (0 °C), acidified to pH 1-2 with concentrated hydrochloric acid and then evaporated to dryness. The residue was mixed with MeOH (100 mL) and the mixture was warmed slightly. The mixture was filtered, and the filtrate was evaporated to give amino acid hydrochloride **37.1** (1.08 g, 100%) as a pale yellow foam: FTIR ($CDCl_3$ cast) 3400-2400 (br), 1689 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 1.30 (s, 9 H), 3.19 (dd, J_{AB} = 12.9 Hz, J_{AX} = 9.5 Hz, 1 H), 3.27-3.30 (m, 1 H), 3.62 (ABq, $\Delta\nu_{AB}$ = 28.8 Hz, J_{AB} = 17.0 Hz, 2 H), 3.81 (s, 3 H), 4.40 (dd, J = 9.6, 6.0 Hz, 1 H), 7.00 (dd, J = 6.8, 1.9 Hz, 2 H), 7.38 (dd, J = 6.8, 2.1 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 31.2 (q′), 31.9 (t′), 44.2 (s′), 46.4 (t′), 56.0 (q′), 63.4 (d′), 115.7 (d′), 125.7 (s′), 131.1 (d′), 162.1 (s′), 168.7 (s′); exact mass *m/z* calcd for $C_{15}H_{23}NNaO_3S$ (M + Na) 320.1296, found 320.1297.

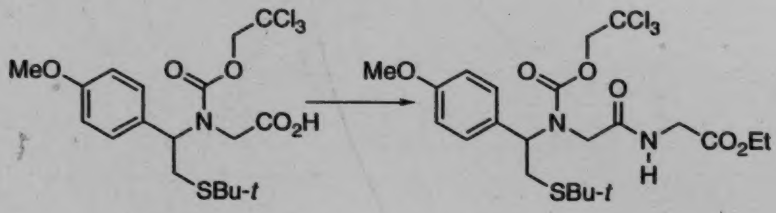
[[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethyl]-(2,2,2-trichloroethoxycarbonyl)amino]acetic Acid (37.2).



37.1 **37.2**

Neat Cl_3CCH_2OCOCl (400 µL, 2.90 mmol) and 1 N NaOH (380 µL) were added alternately in ten equal portions by syringe over 1.5 h to a stirred and cooled (0 °C) suspension of **37.1** (430 mg, 1.45 mmol) in 1 N NaOH (1.70 mL). When addition was complete the cold bath was removed and stirring was continued for 11 h, by which time all **37.1** had reacted. The mixture was cooled (0 °C), acidified to pH 2 with concentrated hydrochloric acid, and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 2:100 and then 4:96 MeOH-CH_2Cl_2, gave **37.2** (510 mg, 74%) as a thick oil: FTIR ($CDCl_3$ cast) 1716, 1611 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) (mixture of rotamers) δ 1.31 (s, 4 H), 1.33 (s, 5 H), 3.01-3.15 (m, 2 H), 3.67-3.83 (m containing a singlet at δ 3.77, 5 H in all), 4.73-4.81 (m, 1 H), 4.87 (ABq, Δv_{AB} = 92.0 Hz, J_{AB} = 11.9 Hz, 2 H), 5.43-5.50 (m, 1 H), 6.84-6.87 (m, 2 H), 7.21-7.24 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) (mixture of rotamers) δ 29.1 (s'), 29.6 (s'), 30.9 (q'), 43.0 (t'),43.2 (t'), 44.9 (t'), 45.2 (t'), 55.3 (q'), 59.6 (d'), 60.0 (d'), 75.4 (t'), 75.6 (t'), 95.1 (s'), 95.2 (s'), 114.2 (d'), 114.3 (d'), 128.4 (s'), 128.5 (d'), 129.4 (d'), 129.6 (d'), 129.7 (d'), 129.8 (d'), 154.2 (s'), 154.4 (s'), 159.6 (s'), 173.5 (s'), 174.1 (s'); exact mass *m/z* calcd. for $C_{18}H_{24}Cl_3NNaO_5S$ (M + Na) 494.0338, found 494.0338.

[2-[[2-tert-Butylsulfanyl-1-(4-methoxyphenyl)-ethyl](2,2,2-trichloroethoxycarbonyl)amino]acetylamino]acetic Acid Ethyl Ester (37.3).



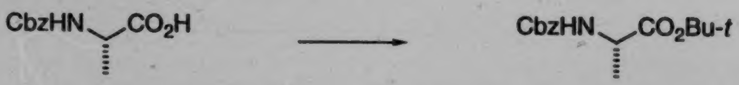
37.2 **37.3**

Ethyl glycinate hydrochloride (5.2 g, 38.6 mmol) was mixed with solid K_2CO_3 (10 g, 72.4 mmol) and a few drops of saturated aqueous NaCl and the mixture was ground with a pestle and mortar to form a thick paste. This was extracted with Et_2O, and the combined extracts were dried ($MgSO_4$) and evaporated. The resulting crude $H_2NCH_2CO_2Et$ was distilled (60 °C, water pump) to afford pure (^1H NMR) $H_2NCH_2CO_2Et$ (2.3 g, 62%).

EDCI (207 mg, 1.08 mmol) was added to a stirred and cooled (0 °C) solution of acid **37.2** (510 mg, 1.08 mmol) and $H_2NCH_2CO_2Et$ (110 μL, 1.08 mmol) in dry CH_2Cl_2 (12 mL). After 15 min the cold bath was removed and stirring was continued for 2 h. The mixture was diluted with Et_2O (100 mL), washed with water (2 x 25 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 1:99, 2:98 and then 4:96 MeOH-CH_2Cl_2, gave **37.3** (374 mg, 62%) as a gum: FTIR ($CDCl_3$ cast) 3323 (br), 1747, 1716,

1611 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) (mixture of rotamers) δ 1.24 (t, *J* = 7.1 Hz, 3 H), 1.32 (br s, 9 H), 3.04-3.29 (br m, 2 H), 3.64-3.76 (br, 2 H), 3.76 (s, 3 H), 3.81-3.96 (m, 2 H), 4.17 (q, *J* = 7.1 Hz, 2 H), 4.74-4.83 (br m, 1.6 H), 5.00-5.10 (br m, 0.4 H), 5.32-5.56 (br m, 1 H), 6.70-6.80 (br s, 1 H), 6.80-6.94 (m, 2 H), 7.21-7.30 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) (mixture of rotamers) δ 14.1 (q′), 29.7 (t′), 30.9 (q′), 41.2 (t′), 42.9 (t′), 47.7 (t′), 55.2 (q′), 59.7 (d′), 60.4 (d′), 61.4 (t′), 75.5 (s′), 95.2 (s′), 114.2 (d′), 129.1 (d′), 129.6 (s′), 154.6 (s′), 159.6 (s′), 168.8 (s′), 169.2 (s′); exact mass *m/z* calcd for $C_{22}H_{31}Cl_3N_2NaO_6S$ (M + Na) 579.0866, found 579.0868.

(*S*)-2-(Benzyloxycarbonylamino)propionic Acid *tert*-Butyl Ester (38.3).

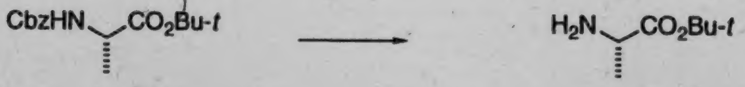


38.2 **38.3**

A solution of *t*-butyl trichloroacetimidate [$Cl_3CC(=NH)$-OBu-*t*] (13.8 g, 63.2 mmol) in dry cyclohexane (distilled from CaH_2) (60.9 mL) was added over 10 min to a stirred and cooled (0 °C) solution of **38.2** (7.05 g, 31.6 mmol) in dry CH_2Cl_2 (30.4 mL), followed by $BF_3.OEt_2$ (610 μL, 4.81 mmol), which was also added over ca 10 min. Stirring was continued for 14 h and the mixture was neutralized with solid $NaHCO_3$ (5 g).

Evaporation of the solvent and flash chromatography of the residue over silica gel (2 x 20 cm), using 1:9 acetone-hexanes, gave **38.3** (7.72 g, 87%) as a colorless oil: $[\alpha]^{20}_{D}$ -28.4° (*c* 1, EtOH); FTIR ($CHCl_3$ cast) 3339, 1723 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (d, *J* = 7.1 Hz, 3 H), 1.44 (s, 9 H), 4.20-4.27 (m, 1 H), 5.05-5.12 (m, 2 H), 5.30-5.41 (br m, 1 H), 7.28-7.34 (m, 5 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.8 (q'), 27.9 (q'), 50.1 (d'), 66.7 (t'), 81.8 (s'), 128.0 (d'), 128.3 (d'), 128.4 (d'), 136.4 (s'), 156.5 (s'), 172.1 (s'); exact mass *m/z* calcd for $C_{15}H_{21}NNaO_4$ (M + Na) 302.1363, found 302.1362.

(2*S*)-2-Aminopropionic Acid *tert*-Butyl Ester (38.4).

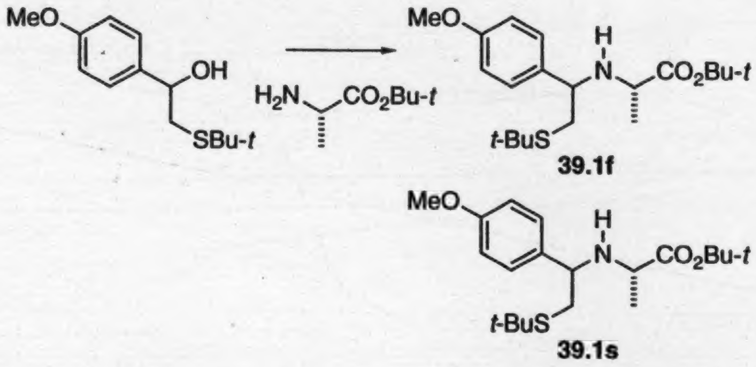


38.3 **38.4**

10% Pd-C (778 mg, 25% weight of **38.3**) was added slowly, under a continuous stream of N_2, to a solution of carbamate **38.3** (3.11 g, 7.84 mmol) in dry MeOH (60 mL). The flask was purged with H_2 gas and the mixture was stirred under a H_2 atmosphere, using a balloon. After 6 h, the mixture was filtered through a Celite pad (3 x 5 cm), using MeOH (25 mL). The combined filtrate and washings were carefully evaporated (the product is volatile) to yield amine **38.4** (1.84 g, 89%)

as a pale yellow oil: $[\alpha]^{20}{}_D$ +2.3° (*c* 1, $CHCl_3$); FTIR ($CHCl_3$ cast) 1731 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.29 (d, *J* = 7.0 Hz, 3 H), 1.43 (s, 9 H), 2.33 (br s, 2 H), 3.43 (q, *J* = 7.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.4 (q'), 28.0 (q'), 50.6 (d'), 81.0 (s'), 175.1 (s'); exact mass *m/z* calcd for $C_7H_{16}NO_2$ (M + H) 146.1181, found 146.1183. Examination of the Mosher amide showed (^{19}F NMR) showed no epimerization.

(2*S*)-2-[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)-ethylamino]propionic Acid *tert*-Butyl Ester (less polar isomer) (39.1f) and (2*S*)-2-[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethylamino]propionic Acid *tert*-Butyl Ester (more polar isomer) (39.1s).

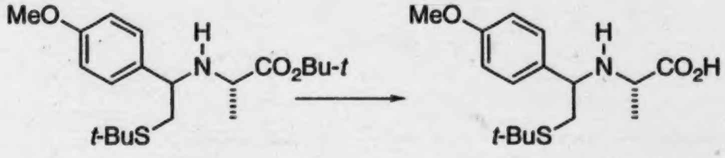


24.1 **39.1f & 39.1s**

Me_3SiBr (295 μL, 2.23 mmol) was added dropwise to a stirred and cooled (0 °C) solution of alcohol **24.1** (537 mg, 2.23 mmol) in dry CH_2Cl_2 (11 mL). After 20 min freshly prepared amine **38.4** (see conversion of **38.3** to **38.4**) (653 mg, 4.47 mmol) was added in one portion. The cold bath was left in place but was not recharged, and stirring was continued for 2 days. Evaporation of the solvent and flash chromatography of the residue over silica gel (3 x 20 cm), using 1:7 EtOAc-hexanes, gave a fast-eluting diastereoisomer **39.1f** (262 mg, 31.8%) as a pale yellow oil and a slow-eluting diastereoisomer **39.1s** (251 mg, 30.5%) as a colorless oil. Isomer **39.1f** had: $[\alpha]^{20}_D$ -89.8° (*c* 1.0, $CHCl_3$); FTIR ($CHCl_3$ cast) 1728 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.17 (d, J = 7.0 Hz, 3 H), 1.30 (s, 9 H), 1.45 (s, 9 H), 2.46-2.54 (br s, 1 H), 2.66 (dd, J_{AB} = 11.7 Hz, J_{AX} = 9.5 Hz, 1 H), 2.76 (dd, J_{AB} = 12.1 Hz, J_{BX} = 4.2 Hz, 1 H), 2.93 (q, J = 7.1 Hz, 1 H), 3.67 (dd, J_{AX} = 9.5 Hz, J_{BX} = 4.2 Hz, 1 H), 3.78 (s, 3 H), 6.83-6.86 (m, 2 H), 7.22-7.25 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 19.7 (q'), 28.2 (q'), 31.0 (q'), 37.4 (t'), 42.4 (s'), 54.7 (d'), 55.3 (q'), 60.2 (d'), 80.6 (s'), 113.9 (d'), 128.3 (d'), 134.9 (s'), 158.9 (s'), 174.9 (s'); exact mass *m/z* calcd for $C_{20}H_{33}NNaO_3S$ (M + Na) 390.2079, found 390.2074.

Isomer **39.1s** had: $[\alpha]^{20}_D$ + 6.3° (*c* 1.0, $CHCl_3$); FTIR ($CHCl_3$ cast) 1729 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.22 (d, J = 6.9 Hz, 3 H), 1.27 (s, 9 H), 1.37 (s, 9 H), 2.80 (br d, J = 5.5 Hz, 2 H), 3.20 (q, J = 6.8 Hz, 1 H), 3.72-3.76 [overlapping signals containing a multiplet and a singlet at δ 3.74, 4 H in all), 6.82 (d, J = 8.6 Hz, 2 H), 7.21 (d, J = 8.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.4 (q'), 28.1 (q'), 31.1 (q'), 35.9 (t'), 42.4 (s'), 54.7 (d'), 55.3 (q'), 60.1 (d'), 80.9 (s'), 113.9 (d') 128.4 (d'), 158.9 (s'); exact mass *m/z* calcd for $C_{20}H_{33}NNaO_3S$ 390.2079 (M + Na), found 390.2079.

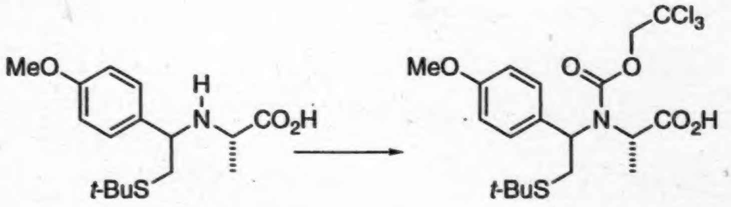
(2*S*)-2-[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)-ethylamino]propionic Acid (less polar isomer) (39.2f).



39.1f **39.2f**

CF_3CO_2H (2 mL) was added to a stirred and cooled (0 °C) mixture of **39.1f** (315 mg, 0.856 mmol) and PhOMe (150 μL), and stirring was continued for 5 h at 0 °C. Evaporation of the solvents and flash chromatography of the residue over silica gel (2 x 15 cm), using 8:92 and then 1:1 MeOH-CH_2Cl_2, gave **39.2f** (264 mg, 99%) as a white solid: $[\alpha]^{20}_D$ -18.1° (*c* 1.0, MeOH); FTIR (MeOH cast) 1678 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 1.25-1.28 (overlapping signals containing a singlet at δ 1.26, 12 H in all), 1.90 (br s, 1 H), 3.01 (dd, J_{AB} = 13.1 Hz, J_{AX} = 8.2 Hz, 1 H), 3.09-3.16 (m, 2 H), 3.73 (s, 3 H), 4.25-4.31 (m, 1 H), 4.80 (br s, 1 H), 6.89 (d, *J* = 8.8 Hz, 2 H), 7.21 (d, *J* = 8.8 Hz, 2 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 17.0 (q′), 31.3 (q′), 32.7 (s′), 44.1 (t′), 49.6 (d′), 55.9 (q′), 63.2 (d′), 115.8 (d′), 126.7 (s′), 131.0 (d′), 162.3 (s′), 173.9 (s′); exact mass *m/z* calcd for $C_{16}H_{25}NNaO_3S$ (M + Na) 334.1453, found 334.1450.

(2*S*)-2-[[2-tert-Butylsulfanyl-1-(4-methoxyphenyl)ethyl](2,2,2-trichloroethoxycarbonyl)amino]-propionic Acid (39.3f).

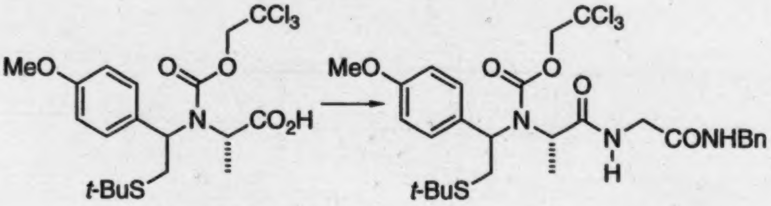


39.2f **39.3f**

Neat Cl_3CCH_2OCOCl (280 μL, 2.03 mmol) and 1 N NaOH (263 μL) were added simultaneously by syringe over 4 h to a stirred and cooled (0 °C) suspension of **39.2f** (316 mg, 1.01 mmol) in 1 N NaOH (1.18 mL). When addition was complete the cold bath was removed and stirring was continued for 11 h, by which time all **39.2f** had reacted. The mixture was cooled (0 °C), acidified to pH 2 with concentrated hydrochloric acid, and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 1:99, 2:99 and then 4:96 MeOH-CH_2Cl_2, gave **39.3f** (349 mg, 71%) as a white foam: $[\alpha]^{20}_D$ -61.3° (*c* 1.0, MeOH); mp 58-61 °C; FTIR (MeOH cast) 1713 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) (mixture of rotamers) δ 1.34 (s, 9 H), 1.56 (d, *J* = 6.6 Hz, 1 H), 1.63 (d, *J* = 6.9 Hz, 1 H), 3.13-3.19 (m, 2 H), 3.76 (s, 3 H), 3.82-3.88 (m, 1 H), 4.61 (d, *J* = 11.8 Hz, 0.35 H), 4.77 (d, *J* = 12.9 Hz, 0.33 H), 4.91-4.98 (m, 1 H), 5.45-5.49 (m, 1

H), 6.82-6.86 (m, 2 H), 7.30-7.36 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.9 (q′), 17.2 (q′), 29.7 (t′), 30.1 (t′), 30.9 (q′), 42.9 (s′), 43.0 (s′), 51.6 (d′), 52.2 (d′), 55.2 (q′), 60.0 (d′), 60.8 (d′), 75.1 (t′), 75.5 (t′), 94.7 (s′), 95.3 (s′), 113.8 (d′), 114.1 (d′), 128.4 (s′), 129.8 (d′), 130.0 (d′), 152.9 (s′), 153.9 (s′), 159.4 (s′), 175.9 (s′), 176.4 (s′); exact mass *m/z* calcd for $C_{19}H_{26}Cl_3NNaO_5S$ (M + Na) 508.0495, found 508.0495.

[(1*S*)-1-[(Benzylcarbamoylmethyl)carbamoyl]ethyl]-[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethyl]-carbamic Acid 2,2,2-Trichloroethyl Ester (39.4f).

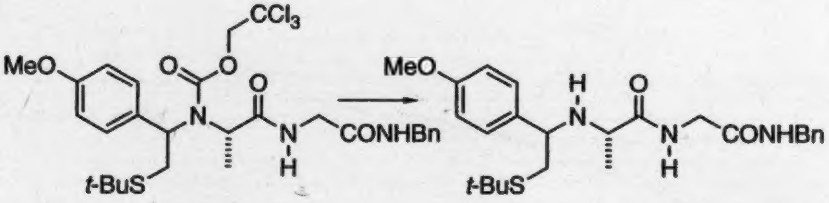


39.3f **39.4f**

HBTU (230 mg, 0.713 mmol) was added to a stirred mixture of acid **39.3f** (334 mg, 0.686 mmol), Et_3N (288 μL, 2.06 mmol) and the amine salt $CF_3CO_2H.H_2NCH_2CONHBn$[45] (198 mg, 0.713 mmol) in MeCN (3 mL). The mixture was stirred for 4 h, diluted with EtOAc (25 mL) and washed successively with 1 N hydrochloric acid (2 x 15 mL) and saturated aqueous $NaHCO_3$ (2 x 15 mL). The organic phase was dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica

gel (2 x 15 cm), using 4:96 MeOH-CH_2Cl_2, gave **39.4f** (345 mg, 79%) as a white crystalline solid: $[\alpha]^{20}_D$ -51.4° (*c* 1.0, $CHCl_3$); mp 61-63 °C; FTIR ($CHCl_3$ cast) 1694, 1666 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.34 (s, 9 H), 1.55 (d, *J* = 6.9 Hz, 3 H), 3.01-3.08 (m, 1 H), 3.15-3.32 (m, 2 H), 3.66-3.79 (overlapping signals containing a singlet at δ 3.78, 4 H in all), 4.05 (dd, *J* = 17.7, 6.8 Hz, 1 H), 4.25 (dd, *J* = 15.3, 4.5 Hz, 1 H), 4.36-4.55 (m, 2 H), 5.24-540 (m, 1 H), 6.03 (br s, 0.7 H), 6.4 (br s, 0.3 H), 6.90 (d, *J* = 7.6 Hz, 2 H), 7.00 (br s, 1 H), 7.22-7.32 (overlapping signals, 7 H in all); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 15.1 (q'), 30.2 (s'), 31.0 (q'), 54.8 (t'), 55.4 (t'), 61.9 (q'), 74.9 (t'), 95.0 (s'), 114.5 (d'), 127.3 (d'), 127.8 (d'), 128.6 (d'), 128.9 (d'), 129.4 (s'), 138.0 (s'), 153.0 (s'), 159.9 (s'), 168.5 (s'), 170.5 (s'); exact mass *m/z* calcd for $C_{28}H_{36}Cl_3N_3NaO_5S$ (M + Na) 654.1339, found 654.1337.

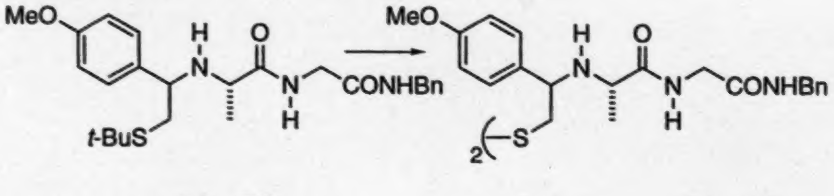
(2*S*)-*N*-(Benzylcarbamoylmethyl)-2-[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethylamino]propionamide (39.5f).



39.4f **39.5f**

Cd powder (880 mg, 7.87 mmol) was added in one portion to a stirred solution of **39.4f** (173 mg, 0.275 mmol) in 1:1 DMF-AcOH (6 mL). Stirring was continued for 3 h at room temperature, and the mixture was filtered through a Celite pad (2 x 4 cm), using EtOAc (50 mL). The combined filtrates and washings were evaporated, and flash chromatography of the residue over silica gel (2 x 20 cm), using 5:100 MeOH-CH_2Cl_2, gave **39.5f** (114 mg, 74%) as a white solid: $[\alpha]^{20}_D$ -49.8° (*c* 1.0, $CHCl_3$); FTIR ($CHCl_3$ cast) 3304, 1655 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.13 (d, *J* = 7.0 Hz, 3 H), 1.26 (s, 9 H), 2.62-2.75 (m, 2 H), 3.00 (q, *J* = 7.0 Hz, 1 H), 3.49 (dd, *J* = 9.2, 5.1 Hz, 1 H), 3.76 (s, 3 H), 3.88-4.04 (m, 2 H), 4.35-4.48 (m, 2 H), 4.59 (br s, 1 H), 6.81-6.85 (m, 2 H), 6.91 (br t, *J* = 5.0 Hz, 1 H), 7.03-7.07 (m, 2 H), 7.19-7.30 (m, 5 H), 8.19 (br t, *J* = 5.9 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.0 (q'), 30.9 (q'), 36.5 (t'), 42.6 (s'), 43.4 (t'), 43.5 (t'), 55.2 (q'), 61.8 (d'), 114.1 (d'), 127.3 (d'), 127.4 (d'), 127.5 (d'), 133.9 (s'), 137.9 (s'), 159.0 (s'), 169.2 (s'), 176.2 (s'); exact mass *m/z* calcd for $C_{25}H_{36}N_3O_3S$ (M + H) 458.2477, found 458.2480.

***N*-(Benzylcarbamoylmethyl)-2-[2-[2-[1-[(benzylcarbamoylmethyl)carbamoyl]ethylamino]-2-(4-methoxyphenyl)ethyldisulfanyl]-1-(4-methoxyphenyl)ethylamino]propionamide (39.6f).**

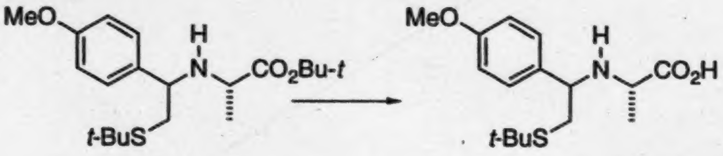


39.5f **39.6f**

In this experiment the initial thiol product was not protected from air, and the compound isolated was the corresponding disulfide **39.6f**.

CF_3CO_2H (1.0 mL) was added to thioether **39.5f** (94.4 mg, 0.207 mmol) contained in a flask immersed in an ice-bath. The mixture was stirred and anisole (40 μL), followed by $Hg(OAc)_2$ (66.0 mg, 0.207 mmol) were added. Stirring was continued for 25 min and the solvent was evaporated. The residue was dissolved in MeCN (15 mL) and H_2S gas was bubbled through the solution for 2 min. The resulting black suspension was filtered through a tightly packed Celite column (2 x 4 cm) and the solid was washed with several portions of MeCN. Evaporation of the combined filtrate and washings, and flash chromatography of the residue over silica gel (2 x 18 cm), using 4:100 $MeOH-CH_2Cl_2$, gave **39.6f** (80.8 mg, 97%) as a pale brown oil: ^1H NMR ($CDCl_3$, 500 MHz) δ 1.17 (d, *J* = 7.0 Hz, 6 H), 1.43 (br s, 2 H), 1.80 (br s, 2 H), 2.59-2.73 (m, 4 H), 2.97-3.05 (m, 2 H), 3.49 (dd, J = 5.7 Hz, 2 H), 3.76 (s, 6 H), 3.91 (dd, J_{AB} = 16.1 Hz, J_{AX} = 5.5 Hz, 2 H), 3.98 (dd, J_{AB} = 16.1 Hz, J_{BX} = 6.1 Hz, 2 H), 4.42 (d, J = 5.7 Hz, 4 H), 6.70 (br s, 2 H), 6.78-6.83 (m, 4 H), 7.01-7.03 (m, 4 H), 7.18-7.28 (m, 10 H), 7.92 (t, J = 5.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.1 (q'), 31.0 (d'), 32.3. (t'), 43.3 (t'), 43.6 (t'), 55.3 (q'), 63.2 (d'), 114.1 (d'), 127.3 (d'), 127.4 (d'), 127.6 (d'), 127.7 (d'), 127.8 (d'), 128.6 (d'), 133.0 (s'), 137.8 (s'), 159.1 (s'), 168.8 (s'), 175.8 (s'); exact mass *m/z* calcd for $C_{42}H_{53}N_6O_6S_2$ (M + H) 801.3462, found 801.3467.

(2*S*)-2-[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)-ethylamino]propionic Acid (41.1s).

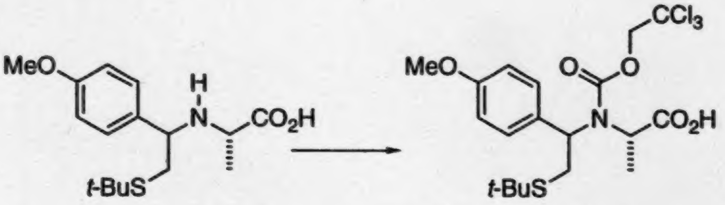


39.1s **41.1s**

CF_3CO_2H (1 mL) was added to a stirred and cooled (0 °C) mixture of **39.1s** (375 mg, 1.02 mmol) and PhOMe (190 μL), and stirring was continued for 5 h at 0 °C. Evaporation of the solvents and flash chromatography of the residue over silica gel (2 x 15 cm), using 8:92 and then 1:1 MeOH-CH_2Cl_2, gave **41.1s** (318 mg, 100%) as a white solid: $[\alpha]^{20}_D$ -4.7° (*c* 1, MeOH); mp 137-142 °C; FTIR (MeOH cast) 1678, 1613 cm^{-1}; ^{1}H NMR

(CD_3OD, 500 MHz) δ 1.28 (s, 9 H), 1.44 (d, J = 7.1 Hz, 3 H), 3.19 (dd, J_{AB} = 12.3 Hz, J_{AX} = 10.1 Hz, 1 H), 3.23 (dd, J_{AB} = 12.6 Hz, J_{BX} = 4.9 Hz, 1 H), 3.45-3.50 (m, 1 H), 3.79 (s, 3 H), 4.29 (dd, J_{AX} = 9.8 Hz, J_{BX} = 5.4 Hz, 1 H), 6.95-6.96 (m, 2 H), 7.34-7.37 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 15.4 (q′), 31.2 (q′), 31.9 (t′), 43.9 (s′), 49.5 (d′), 55.8 (q′), 61.7 (d′), 115.4 (d′), 126.8 (s′), 130.8 (d′), 161.9 (s′); exact mass *m/z* calcd for $C_{16}H_{25}NNaO_3S$ (M + Na) 334.1453, found 334.1454.

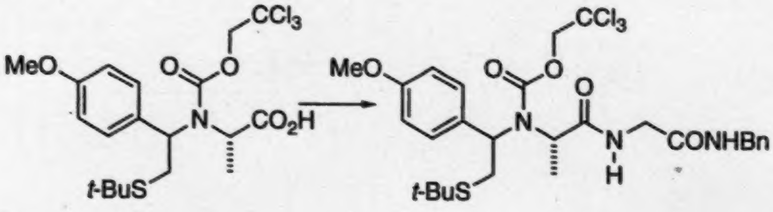
(2*S*)-2-[[-2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethyl](2,2,2-trichloroethoxycarbonyl)amino]-propionic Acid (41.2s).



41.1s **41.2s**

Neat Cl_3CCH_2OCOCl (260 μL, 1.88 mmol) and 1 N NaOH (245 μL) were added simultaneously by syringe over 4 h to a stirred and cooled (0 °C) suspension of **41.1s** (294 mg, 0.942 mmol) in 1 N NaOH (1.10 mL). When addition was complete the cold bath was removed and stirring was continued for 11 h, by which time all **41.1s** had reacted. The mixture was cooled (0 °C), acidified to pH 2 with concentrated hydrochloric acid, and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 1:99, 2:99 and then 4:96 MeOH-CH_2Cl_2, gave **41.2s** (337 mg, 73%) as a white foam: $[\alpha]^{20}_{D}$ -0.9° (*c* 1.0, MeOH); mp 64-65 °C; FTIR (MeOH cast) 1712, 1611 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) (mixture of rotamers) δ 0.97 (d, *J* = 6.8 Hz, 1.1 H), 1.03 (d, *J* = 6.8 Hz, 1.5 H), 1.33 (s, 3.8 H), 1.35 (s, 5.2 H), 3.08-3.18 (m, 2 H), 3.67-3.74 (m, 1 H), 3.79 (s, 3 H), 4.69-4.75 (m, 1 H), 4.87-5.02 (m, 1 H), 4.94 (ABq, $\Delta\nu_{AB}$ = 48.2 Hz, J_{AB} = 12.0 Hz, 1 H), 5.54-5.61 (m, 1 H), 6.87-6.89 (m, 2 H), 7.24-7.27 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) (mixture of rotamers) δ 14.5 (q'), 16.0 (q'), 28.4 (s'), 28.9 (s'), 30.8 (q'), 43.1 (t'), 43.4 (t'), 52.1 (d'), 52.8 (d'), 55.2 (q'), 59.1 (d'), 59.5 (d'), 75.1 (d'), 75.5 (d'), 94.7 (s'), 95.3 (s'), 114.1 (d'), 128.4 (s'), 129.6 (d'), 129.7 (d'), 152.8 (s'), 153.9 (s'), 159.6 (s'), 175.0 (s'), 175.3 (s'); exact mass *m/z* calcd for $C_{19}H_{26}Cl_3NNaO_5S$ (M + Na) 508.0495, found 508.0495.

[(1*S*)-1-[(Benzylcarbamoylmethyl)carbamoyl]ethyl]-[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethyl]-carbamic Acid 2,2,2-Trichloroethyl Ester (41.3s).

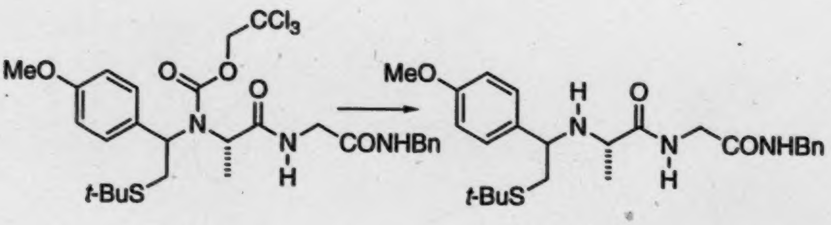


41.2s **41.3s**

HBTU (186 mg, 0.556 mmol) was added to a stirred mixture of acid **41.2s** (271 mg, 0.578 mmol), Et_3N (233 µL, 1.67 mmol) and the amine salt $CF_3CO_2H.H_2NCH_2CONHBn$[45] (161 mg, 0.578 mmol) in MeCN (3 mL). The mixture was stirred for 4 h, diluted with EtOAc (25 mL) and washed successively with 1 N hydrochloric acid (2 x 15 mL) and saturated aqueous $NaHCO_3$ (2 x 15 mL). The organic phase was dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 4:96 MeOH-CH_2Cl_2, gave **41.3s** (314 mg, 89%) as a white crystalline solid: $[\alpha]^{20}_D$ +26.9° (*c* 1.0, $CHCl_3$); mp 74-76 °C; FTIR ($CHCl_3$ cast) 1695, 1611 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.83 (d, *J* = 6.8 Hz, 3 H), 1.35 (s, 9 H), 3.05 (dd, J_{AB} = 11.9 Hz, J_{AX} = 3.5 Hz, 1 H), 3.21 (dd, J_{AB} = J_{BX} = 11.9 Hz, 1 H), 3.58 (dd, *J* = 17.1, 5.0 Hz, 1 H), 3.66 (m, 1 H), 3.78 (s, 3 H), 3.99 (d, *J* = 12.0 Hz, 1 H), 4.19 (dd, *J* = 14.9, 4.3 Hz, 1 H), 4.39 (dd, *J* = 17.1, 7.6 Hz, 1 H), 4.54 (d, *J* = 12.0 Hz, 1 H), 4.67 (dd, *J* = 14.8, 7.2 Hz, 1

H), 5.61 (dd, J = 11.7, 3.3 Hz, 1 H), 6.85-6.87 (m, 2 H), 7.20-7.33 (m, 7 H), 7.33-7.35 (m, 1 H), 7.91 (br t, J = 6.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0 (q′), 29.8 (s′), 43.0 (t′), 43.2 (t′), 53.2 (d′), 55.4 (q′), 57.7 (d′), 74.8 (t′), 95.1 (s′), 114.4 (d′), 127.4 (d′), 127.9 (d′), 128.6 (d′), 128.9 (d′), 129.4 (s′), 138.4 (s′), 153.4 (s′), 159.9 (s′), 169.1 (s′), 171.3 (s′); exact mass *m/z* calcd for $C_{28}H_{36}Cl_3N_3NaO_5S$ (M + Na) 654.1339, found 654.1339.

(2*S*)-*N*-(Benzylcarbamoylmethyl)-2-[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethylamino]propionamide (41.4s).

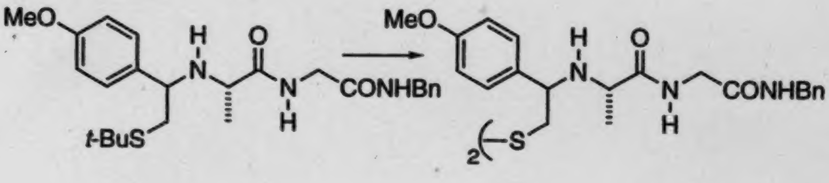


41.3s **41.4s**

Cd powder (1.32 g, 11.7 mmol) was added in one portion to a stirred solution of **41.3s** (258 mg, 0.410 mmol) in 1:1 DMF-AcOH (9 mL). Stirring was continued for 5 h at room temperature, and the mixture was filtered through a Celite pad (2 x 4 cm), using EtOAc (50 mL). The combined filtrates and washings were evaporated and flash chromatography of the residue over silica gel (2 x 20 cm), using 5:100 MeOH-CH_2Cl_2, gave **41.4s** (258 mg, 87%) as a glassy liquid: $[\alpha]^{20}{}_D$ +18.0°

(*c* 1.0, $CHCl_3$); FTIR (CH_2Cl_2 cast) 3304, 1652, 1610 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.24 (d, J = 7.1 Hz, 3 H), 1.30 (s, 9 H), 2.11 (br s, 1 H), 2.68 (dd, J_{AB} = 12.5 Hz, J_{AX} = 10.0 Hz, 1 H), 2.78 (dd, J_{AB} = 12.6 Hz, J_{BX} = 3.8 Hz, 1 H), 2.90-2.98 (m, 1 H), 3.65-3.81 (m containing a singlet at δ3.70, 5 H in all), 4.32 (dd, J_{AB} = 14.9 Hz, J_{AX} = 5.7 Hz, 1 H), 4.39 (dd, J_{AB} = 14.8 Hz, J_{BX} = 5.9 Hz, 1 H), 6.75-6.78 (m, 2 H), 6.81-6.85 (m, 1 H), 7.17-7.28 (m, 7 H), 7.94 (br t, J = 5.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.9 (q'), 31.0 (q'), 36.6 (s'), 42.7 (t'), 43.1 (t'), 43.2 (t'), 55.1 (q'), 55.5 (d'), 61.2 (d'), 114.0 (d'), 127.3 (d'), 127.4 (d'), 127.6 (d'), 128.3 (d'), 128.6 (d'), 134.2 (s'), 137.9 (s'), 159.2 (s'), 168.8 (s'), 175.6 (s'); exact mass *m/z* calcd for $C_{25}H_{36}N_3O_3S$ (M + H) 458.2477, found 458.2476.

***N*-(Benzylcarbamoylmethyl)-2-[2-[2-[1-[(benzylcarbamoylmethyl)carbamoyl]ethylamino]-2-(4-methoxyphenyl)ethyldisulfanyl]-1-(4-methoxyphenyl)ethylamino]propionamide (41.5s).**

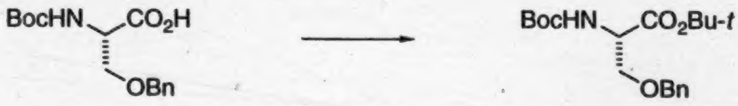


41.4s **41.5s**

In this experiment the initial thiol product was not protected from air, and the compound isolated was the corresponding disulfide **41.5s**.

CF_3CO_2H (1.0 mL) was added to thioether **41.4s** (156 mg, 0.342 mmol) contained in a flask immersed in an ice-bath. The mixture was stirred and PhOMe (62 μL), followed by $Hg(OAc)_2$ (109 mg, 0.342 mmol) were added. Stirring was continued for 25 min and the solvent was evaporated. The residue was dissolved in MeCN (15 mL) and H_2S gas was bubbled through the solution for 2 min. The resulting black suspension was filtered through a tightly packed Celite column (2 x 4 cm) and the solid was washed with several portions of MeCN. Evaporation of the combined filtrate and washings, and flash chromatography of the residue over silica gel (2 x 18 cm), using 4:100 MeOH-CH_2Cl_2, gave **41.5s** (124 mg, 73%) as a pale brown oil: $[\alpha]^{20}_D$ -7.0 (c 0.74, MeOH); FTIR (MeOH, cast) 3291, 1651 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 1.31 (d, J = 7.0 Hz, 6 H), 2.72-2.83 (m, 4 H), 3.13-3.20 (m, 2 H), 3.71-3.83 (m containing a singlet at δ 3.73, 12 H in all), 4.37 (s, 4 H), 6.81-6.85 (m, 4 H), 7.18-7.29 (m, 14 H); ^{13}C NMR (CD_3OD, 125 MHz) (mixture of rotamers) δ 18.1 (q'), 18.4 (d'), 31.6 (t'), 43.4 (t'), 44.2 (t'), 46.4 (t'), 55.8 (q'), 60.7 (d'), 64.7 (d'), 115.0 (d'), 128.2 (d'), 128.4 (d'), 128.5 (d'), 129.5 (d'), 130.0 (d'), 130.0 (d'), 130.1 (s'), 139.7 (s'), 160.8 (s'), 160.9 (s'), 171.0 (s'); exact mass *m/z* calcd for $C_{42}H_{53}N_6O_6S_2$ (M + H) 801.3462, found 801.3467.

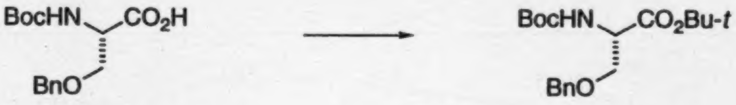
(2*S*)-3-Benzyloxy-2-*tert*-butoxycarbonylamino-propionic Acid *tert*-Butyl ester (42.2).



42.1 **42.2**

DMAP (504 mg, 4.13 mmol) was added in one portion to a stirred solution of **42.1** (4.06 g, 13.8 mmol) and Boc_2O (4.34 g, 19.3 mmol) in dry *t*-BuOH (90 mL). Rapid evolution of gas occurred. After 3 h, the solvent was evaporated and flash chromatography of the residue over silica gel (4 x 25 cm), using 1:9 acetone-hexanes, gave **42.2** (1.98 g, 41%) as a colorless oil: $[\alpha]^{20}_D$ +6.5° (*c* 1, $CHCl_3$). We subsequently found that the material had been partially epimerized, and a better synthetic route is described below.

(2*S*)-3-Benzyloxy-2-(*tert*-butoxycarbonylamino)-propionic Acid *tert*-Butyl Ester (42.2).

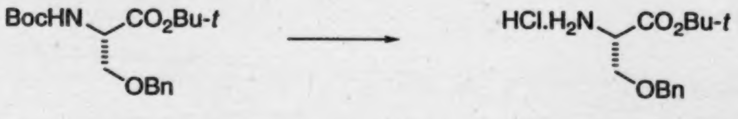


42.1 **42.2**

The following is the best procedure for making the t-butyl ester, as epimerization, if any, appears to be very slight.

A solution of *t*-butyl trichloroacetimidate [$Cl_3CC(=NH)$-OBu-*t*] (13.9 g, 63.6 mmol) in dry cyclohexane (60.9 mL) was added over 10 min to a stirred and cooled (0 °C) solution of **42.1** (8.98 g, 30.4 mmol) in dry CH_2Cl_2 (30.4 mL), followed by $BF_3.OEt_2$ (610 μL, 4.81 mmol), which was also added over ca 10 min. Stirring was continued for 14 h and the mixture was neutralized with solid $NaHCO_3$ (5 g). Evaporation of the solvent and flash chromatography of the residue over silica gel (2 x 20 cm), using 1:9 acetone-hexanes, gave **42.2** (6.76 g, 63%) as a pale yellow oil: $[\alpha]^{20}{}_D$ +8.0° (*c* 1, $CHCl_3$); FTIR (CH_2Cl_2 cast) 1716 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.432 (s, 9 H), 1.435 (s, 9 H), 3.64 (dd, J_{AB} = 9.3 Hz, J_{AX} = 3.1 Hz, 1 H), 3.83 (dd, J_{AB} = 9.3 Hz, J_{BX} = 3.1 Hz, 1 H), 4.28-4.32 (m, 1 H), 4.50 (ABq, $\Delta\nu_{AB}$ = 30.8 Hz, J_{AB} = 12.1 Hz, 2 H), 5.35 (d, *J* = 8.4 Hz, 1 H), 7.24-7.34 (m, 5 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.0 (q′), 28.4 (q′), 54.4 (d′), 70.5 (t′), 73.3 (t′), 79.6 (s′), 81.9 (s′), 127.4 (d′), 127.6 (d′), 128.3 (d′), 137.6 (s′), 155.4 (s′), 169.5 (s′); exact mass *m/z* calcd for $C_{19}H_{29}NNaO_5$ (M + Na) 374.1943, found 374.1945.

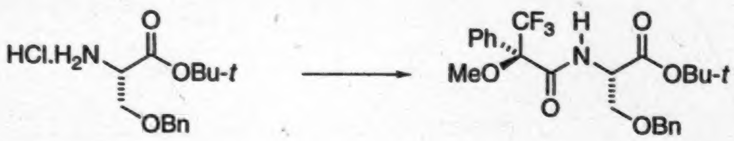
(2*S*)-2-Amino-3-benzyloxypropionic Acid *tert*-Butyl Ester Hydrochloride (42.3).



42.2 **42.3**

Dry HCl gas was passed into cold (0 °C) EtOAc (50 mL) for 20 min and the solution was allowed to warm slowly to room temperature. A portion of this solution (7.5 N[46] 4.49 mL, 33.7 mmol) was added to a stirred solution of **42.2** (made by use of *t*-butyl trichloroacetimidate, 1.99 g, 6.73 mmol) in EtOAc (29.2 mL) contained in a round-bottom flask fitted with a rubber septum, and stirring was continued for 18 h. The resulting precipitate was collected, and recrystallization from MeOH-EtOAc, gave **42.3** (1.09 g, 67%) as a white, fluffy solid: $[\alpha]^{20}_{D}$ -4.3° (*c* 1, MeOH); mp 181-183 °C; FTIR (MeOH cast) 3700-3400 (br), 1736 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 1.48 (s, 9 H), 3.78 (dd, J_{AB} = 10.4 Hz, J_{AX} = 3.1 Hz, 1 H), 3.90 (dd, J_{AB} = 10.4 Hz, J_{BX} = 4.2 Hz, 1 H), 4.13 (dd, J_{AX} = 3.1 Hz, J_{BX} = 4.2 Hz, 1 H), 4.57 (ABq, $\Delta\nu_{AB}$ = 58.3 Hz, J_{AB} = 12.0 Hz, 2 H), 7.26-7.34 (m, 5 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 28.1 (q′), 54.9 (d′), 68.2 (t′), 74.6 (t′), 85.3 (s′), 129.2 (d′), 129.24 (d′), 129.55 (d′), 138.4 (s′), 167.6 (s′); exact mass *m/z* calcd for $C_{14}H_{22}NO_3$ (M + H) 252.1600, found 252.1604.

(2*S*)-3-Benzyloxy-2-[(2*R*)-(3,3,3-trifluoro-2-methoxy-2-phenylpropionylamino)]propionic Acid *tert*-Butyl Ester (Mosher Amide).

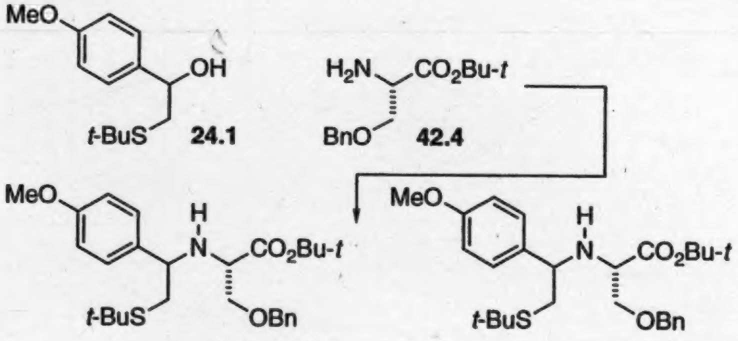


42.3

-

Et_3N (23.1 μL, 0.165 mmol) was added to a stirred and cooled (0 °C) mixture of (-)-α-methoxy-α-trifluoromethylphenyl acetic acid (77.2 mg, 0.330 mmol) and amine hydrochloride **42.3** (47.4 mg, 0.165 mmol) in dry CH_2Cl_2 (1.5 mL), followed by EDCI (64.5 mg, 0.330 mmol). Stirring was continued at 0 °C for 3 h by which time all **42.3** had reacted (tlc control, silica, 1:4 EtOAc-hexanes). Evaporation of the mixture and flash chromatography of the residue over silica gel (1 x 15 cm), using 1:4 EtOAc-hexanes, gave the Mosher amide (72.6 mg, 94.3%) as a colorless oil: $[\alpha]^{20}_D$ -12.1° (*c* 1.0, MeOH); FTIR (MeOH cast) 1678 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.47 (s, 9 H), 3.49 (s, 3 H), 3.62 (dd, *J* = 9.6, 3.1 Hz, 1 H), 3.82 (dd, *J* = 9.6, 3.1 Hz, 1 H), 4.40 (ABq, $\Delta\nu_{AB}$ = 16.1 Hz, J_{AB} = 12.1 Hz, 2 H), 4.63-4.66 (m, 1 H), 7.13-7.15 (m, 2 H), 7.21-7.35 (m, 6 H), 7.44 (br d, *J* = 8.0 Hz, 1 H), 7.50-7.51 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.0 (q′), 53.1 (q′), 55.2 (d′), 69.6 (t′), 73.3 (t′), 82.7 (s′), 83.9 (q, *J* = 26.3 Hz), 122.5 (s′), 124.8 (s′), 127.4 (d′), 127.6 (d′), 127.7 (d′), 128.3 (d′), 129.3 (d′), 132.8 (s′), 137.4 (s′), 165.9 (s′), 168.4 (s′); ^{19}F NMR ($CDCl_3$, 468 MHz) δ -69.52 (s, integral 1.24), -69.08 (s, integral 88.45); exact mass *m/z* calcd for $C_{24}H_{28}F_3NO_5Na$ (M + Na) 490.1812, found 490.1812. The ^{19}F NMR spectrum indicated an ee of 97.2%.

(2*S*)-3-Benzyloxy-2-[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethylamino]propionic Acid *tert*-Butyl Ester (less polar isomer) (43.1f) and (2*S*)-3-Benzyloxy-2-[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)-ethylamino]propionic Acid *tert*-Butyl Ester (more polar isomer) (43.1s).



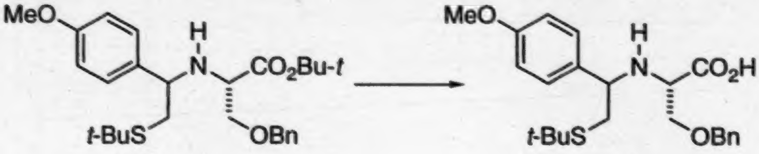
43.1f **43.1s**

Me_3SiBr (390 µL, 2.93 mmol) was added dropwise to a stirred and cooled (0 °C) solution of alcohol **24.1** (704 mg, 2.93 mmol) in dry CH_2Cl_2. After 40 min, a solution of **42.4** (1.47 g, 5.87 mmol) in dry CH_2Cl_2 (3 mL) was injected in one portion. Stirring was continued for 3 h without recharging the cold bath. Evaporation of the solvent and flash chromatography of the residue over silica gel (3.5 x 25 cm), using 4:12:100 *t*-BuOMe-Et_2O-petroleum ether (35-60 °C), gave the faster-eluting diastereoisomer **43.1f** (422 mg, 30%) as a colorless oil. The slower-eluting fraction was resubjected to flash chromatography over silica gel (3.5 x 25 cm), using 1:9 EtOAc-hexanes, to obtain **43.1s** (539 mg, 38%) as a

colorless oil. Isomer **43.1f** had: $[\alpha]^{20}_{D}$ -60.0° (*c* 1.0, CH_2Cl_2); FTIR (CH_2Cl_2 cast) 3311, 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.29 (s, 9 H), 1.44 (s, 9 H), 2.66 (dd, J_{AB} = 12.1 Hz, J_{AX} = 9.3 Hz, 1 H), 2.75 (dd, J_{AB} = 12.2 Hz, J_{BX} = 4.7 Hz, 1 H), 2.72-2.80 (br s, 1 H), 3.08 (t, J = 5.1 Hz, 1 H), 3.49 (dd, J_{AB} = 9.0 Hz, J_{AX} = 5.4 Hz, 1 H), 3.57 (dd, J_{AB} = 9.1 Hz, J_{BX} = 4.7 Hz, 1 H), 3.74 (dd, J_{AX} = 9.2 Hz, J_{BX} = 4.8 Hz, 1 H), 3.76 (s, 3 H), 4.44 (ABq, $\Delta\nu_{AB}$ = 22.1 Hz, J_{AB} = 12.2 Hz, 2 H), 6.79-6.82 (m, 2 H), 7.19-7.28 (m, 7 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.2 (q′), 31.1 (q′), 37.4 (t′), 42.4 (s′), 55.3 (q′), 59.3 (d′), 60.2 (d′), 72.0 (t′), 73.1 (t′), 81.1 (s′), 113.8 (d′), 127.3 (d′), 127.4 (d′), 128.1 (d′), 128.4 (d′), 134.7 (s′), 138.1 (s′), 158.9 (s′), 172.3 (s′); exact mass *m/z* calcd for $C_{27}H_{40}NO_4S$ (M + H) 474.2678, found 474.2674.

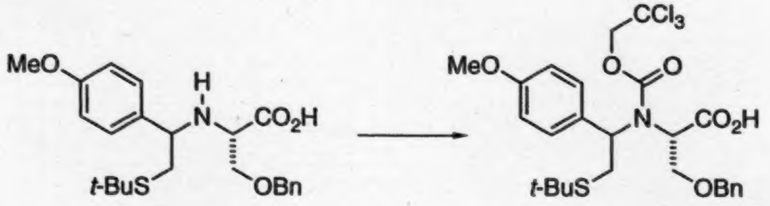
Isomer **43.1s** had: $[\alpha]^{20}_{D}$ +22.2° (*c* 1.0, CH_2Cl_2); FTIR (CH_2Cl_2 cast) 1731 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.27 (s, 9 H), 1.35 (s, 9 H), 2.50 (br s, 1 H), 2.73-2.80 (m, 2 H), 3.31 (t, J = 4.6 Hz, 1 H), 3.56-3.61 (m, 2 H), 3.73-3.61 (overlapping signals containing a singlet at δ 3.74, 4 H in all), 4.49 (ABq, $\Delta\nu_{AB}$ = 14.5 Hz, J_{AB} = 12.1 Hz, 2 H), 6.78-6.81 (m, 2 H), 7.19-7.29 (m, 7 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.1 (q′), 31.2 (q′), 36.4 (t′), 42.3 (s′), 55.3 (q′), 59.6 (d′), 60.4 (d′), 70.3 (t′), 73.3 (t′), 81.0 (s′), 113.8 (d′), 126.9 (d′), 127.5 (d′), 128.2 (d′), 128.4 (d′), 134.7 (s′), 138.0 (s′), 158.9 (s′), 171.9 (s′); exact mass *m/z* calcd for $C_{27}H_{40}NO_4S$ (M + H) 474.2678, found 474.2679.

(2*S*)-3-Benzyloxy-2-[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethylamino]propionic Acid (43.2f).


43.1f **43.2f**

$Me_3SiOSO_2CF_3$ (323 μL, 1.79 mmol) was added dropwise to a stirred and cooled (0 °C) solution of ester **43.1f** (422 mg, 0.893 mmol) in dry CH_2Cl_2 (6.6 mL). Stirring was continued for 6 h without recharging the cold bath and the mixture was applied directly to a silica gel column (2 x 15 cm). Flash chromatography, using 4:100 MeOH-CH_2Cl_2, gave **43.2f** (338 mg, 90%) as a pale yellow solid: $[\alpha]^{20}{}_D$ +2.4° (*c* 1.0, MeOH); mp 172-176 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.28 (s, 9 H), 2.81-2.89 (m, 2 H), 3.32-3.34 (m, 1 H), 3.47 (dd, J = 9.9, 3.9 Hz, 1 H), 3.68-3.82 (m containing a singlet at δ 3.75, 5 H in all), 4.33 (ABq, $\Delta\nu_{AB}$ = 44.7 Hz, J_{AB} = 11.8 Hz, 2 H), 6.76-6.79 (m, 2 H), 7.11-7.30 (m, 7 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 31.0 (q'), 35.6 (s'), 42.9 (t'), 55.3 (q'), 59.5 (d'), 62.5 (d'), 69.2 (t'), 73.0 (t'), 114.2 (d'), 127.6 (d'), 127.7 (d'), 128.2 (d'), 128.3 (d'), 137.3 (s'), 159.5 (s'), 172.3 (s'); exact mass *m/z* calcd for $C_{23}H_{32}NO_4S$ (M + H) 418.2047, found 418.2052.

3-Benzyloxy-2-[[2-tert-butylsulfanyl-1-(4-methoxyphenyl)ethyl](2,2,2-trichloroethoxycarbonyl)-amino]propionic Acid (43.3f).

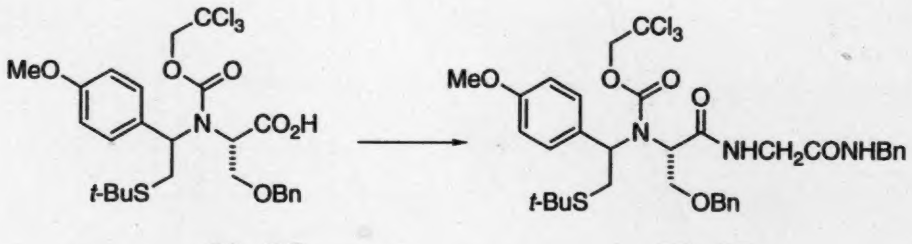


43.2f **43.3f**

A solution of Cl_3CCH_2OCOCl (228 μL, 1.65 mmol) in dioxane (1 mL) and 0.5 N NaOH (430 μL, 215 mmol) were added simultaneously by syringe pump over 4.5 h to a stirred and cooled (0 °C) solution of **43.2f** (308 mg, 0.827 mmol) in 1 N NaOH (0.99 mL). When addition was complete the cold bath was removed and stirring was continued for 14 h. The mixture was diluted with water (5 mL), adjusted to pH 3-4 with 1 N hydrochloric acid, and extracted with Et_2O (2 x 15 mL). The combined extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 25 cm), using 8:100 MeOH-CH_2Cl_2, gave **43.3f** (174 mg, 38%) as a white foam: $[\alpha]^{20}_D$ -34.5° (*c* 1.0, $CHCl_3$); FTIR ($CDCl_3$ cast) 1714 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) (mixture of rotamers) δ 1.22 (s, 3.9 H), 1.28 (s, 5.1 H), 2.93-3.01 (m, 1 H), 3.14-3.24 (m, 1 H), 3.75 (s, 3 H), 3.76-3.80 (m, 1 H), 3.88-3.96 (m, 1 H), 4.04-4.07 (m, 1 H), 4.46-4.58 (m, 2.6 H), 4.70-4.75 (m, 1 H), 4.94 (d, *J* = 11.9 Hz, 0.42 H), 5.42 (dd, *J* = 9.6, 6.4 Hz, 1

H), 6.81 (d, J = 8.5 Hz, 2 H), 7.21-7.32 (m, 7 H); ^{13}C NMR ($CDCl_3$, 125 MHz) (mixture of rotamers) δ 29.6 (t′), 30.2 (t′), 30.9 (q′), 42.7 (s′), 43.0 (s′), 55.2 (q′), 56.7 (d′), 57.4 (d′), 60.4 (d′), 61.4 (d′), 68.3 (t′), 69.2 (t′), 73.5 (t′), 73.6 (t′), 75.1 (t′), 75.5 (t′), 94.6 (s′), 95.3 (s′), 113.7 (s′), 127.6 (s′), 127.62 (s′), 127.7 (d′), 127.8 (d′), 128.4 (d′), 130.6 (d′), 130.7 (d′), 137.4 (s′), 137.5 (s′), 153.2 (s′), 153.5 (s′), 159.3 (s′), 159.4 (s′), 173.5 (s′), 174.0 (s′); exact mass *m/z* calcd for $C_{26}H_{32}Cl_3NNaO_6S$ (M + Na) 614.0908, found 614.0904.

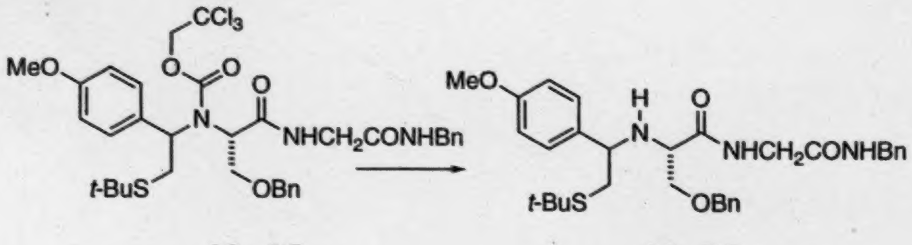
[(2*S*)-1-[(Benzylcarbamoylmethyl)carbamoyl]-2-benzyloxyethyl][2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethyl]carbamic Acid 2,2,2-Trichloroethyl Ester (43.4f).



43.3f **43.4f**

$CF_3CO_2H.H_2NCH_2CONHBn$[45] (92 mg, 0.33 mmol) and Et_3N (134 μL, 0.954 mmol) were added with stirring to dry MeCN (1 mL). Acid **43.3f** (174 mg, 0.318 mmol) and then 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (109 mg, 0.331 mmol) were added. Stirring was continued for 12 h and the mixture was diluted with EtOAc (20 mL) and washed successively with 1 N hydrochloric acid (2 x 15 mL) and saturated aqueous $NaHCO_3$ (2 x 15 mL). The organic phase was dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 1:50 MeOH-Et_2O, gave **43.4f** (168 mg, 71%) as a white foam: $[\alpha]^{20}_D$ -36.5° (*c* 1, $CHCl_3$); FTIR ($CDCl_3$ cast) 3339 (br), 1713, 1692, 1663 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.21 (br s, 9 H), 2.96-3.12 (m, 2 H), 3.42-3.48 (m, 1 H), 3.58-3.88 (m containing singlet at δ 3.74, 6 H in all), 4.20-4.30 (m, 2 H), 4.33-4.54 (m, 4 H), 4.68 (br s, 0.2 H), 5.41-5.47 (m, 1 H), 5.96 (br s, 0.2 H), 6.24 (br s, 0.5 H), 6.60 (br s, 0.3 H), 6.77-6.91 (m, 2 H), 7.19-7.34 (m, 13 H); ^{13}C NMR ($CDCl_3$, 125 MHz) (mixture of rotamers) δ 29.7 (t'), 29.9 (t'), 30.9 (q'), 42.9 (t'), 43.0 (t'), 43.2 (s'), 43.3 (t'), 55.2 (q'), 57.4 (d'), 57.9 (d'), 61.0 (d'), 61.2 (d'), 67.9 (t'), 73.5 (t'), 75.1 (t'), 75.2 (t'), 94.9 (s'), 114.0 (d'), 114.3 (d'), 127.2 (d'), 127.4 (d'), 127.5 (d'), 127.7 (d'), 127.9 (d'), 128.1 (d'), 128.4 (d'), 128.9 (d'), 129.6 (d'), 129.9 (d'), 137.2 (s'), 137.9 (s'), 153.4 (s'), 159.5 (s'), 159.6 (s'), 168.0 (s'), 168.4 (s'), 168.8 (s'), 169.0 (s'); exact mass *m/z* calcd for $C_{35}H_{42}Cl_3N_3NaO_6S$ (M + Na) 760.1752, found 760.1755.

(2*S*)-*N*-(Benzylcarbamoylmethyl)-3-benzyloxy-2-[2-tert-butylsulfanyl-1-(4-methoxyphenyl)ethylamino]-propionamide (43.5f).

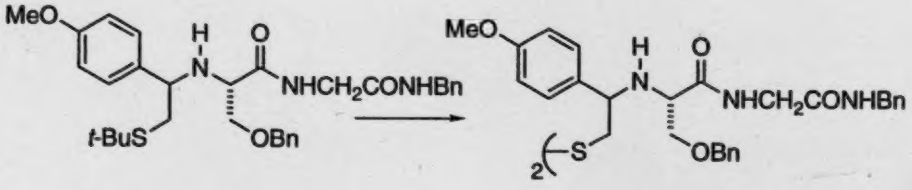


43.4f **43.5f**

Cd powder (591 mg, 5.26 mmol) was added in one portion to a stirred solution of **43.4f** (136 mg, 0.184 mmol) in 1:1 DMF-AcOH (4.0 mL). Stirring was continued for 11 h at room temperature, and the mixture was filtered through a Celite pad (2 x 4 cm), using EtOAc (50 mL). The combined filtrates and washings were evaporated, and the residue was adsorbed onto silica gel (5 g) from MeOH. The solid was applied to the top of a column of silica gel (2 x 20 cm), and flash chromatography, using 1:25 MeOH-CH_2Cl_2, gave **43.5f** (91.6 mg, 88%) as pale yellow resin: $[\alpha]^{20}_D$ -39.0° (*c* 1, MeOH); FTIR ($CDCl_3$ cast) 3306, 1659 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.29 (s, 9 H), 2.63-2.72 (m, 3 H), 3.17 (dd, *J* = 5.7, 3.7 Hz, 1 H), 3.35 (dd, *J* = 9.5, 3.7 Hz, 1 H), 3.51 (dd, *J* = 8.8, 5.4 Hz, 1 H), 3.62 (dd, *J* = 9.4, 5.8 Hz, 1 H), 3.76 (s, 3 H), 3.89 (dd, *J* = 16.5, 5.9 Hz, 1 H), 4.10-4.20 (m, 3 H), 4.27-4.39 (m, 2 H), 6.76 (d, *J* = 8.4 Hz, 2 H), 6.85 (s, 1 H), 7.02 (d, *J* = 8.6 Hz, 2 H), 7.12-7.32 (m, 10 H), 8.44 (t, *J* = 6.5

Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.0 (q′), 36.7 (s′), 42.7 (t′), 43.1 (t′), 43.4 (t′), 55.2 (q′), 59.7 (d′), 62.1 (d′), 71.0 (t′), 72.7 (t′), 114.0 (d′), 127.3 (d′), 127.4 (d′), 127.5 (d′), 127.8 (d′), 128.4 (d′), 128.5 (d′), 134.0 (s′), 137.4 (s′), 138.0 (s′), 159.1 (s′), 169.1 (s′), 173.3 (s′); exact mass *m/z* calcd for $C_{32}H_{42}N_3O_4S$ 564.2890 (M + H), found 564.2896.

(2*S*)-*N*-(Benzylcarbamoylmethyl)-2-[2-[2-[1-[(benzylcarbamoylmethyl)carbamoyl]-2-benzyloxyethylamino]-2-(4-methoxyphenyl)ethyldisulfanyl]-1-(4-methoxyphenyl)ethylamino]-3-benzyloxypropionamide (43.6f).



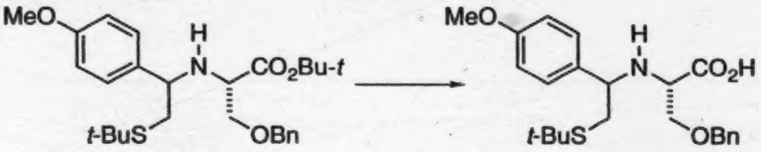
43.5f **43.6f**

In this experiment the initial thiol product was not protected from air, and the compound isolated was the corresponding disulfide **43.6f**. We did not establish if oxidation occurred before or after flash chromatography.

CF_3CO_2H (2 mL) was added to thioether **43.5f** (85.0 mg, 0.153 mmol) contained in a flask immersed in an ice-bath. The mixture was stirred and PhOMe (32 μL), followed by

$Hg(OAc)_2$ (50.5 mg, 0.158 mmol) were added. Stirring was continued for 25 min and the solvent was evaporated. The residue was dissolved in MeCN (20 mL) and H_2S gas was bubbled through the solution for 2 min. The resulting black suspension was filtered through a tightly packed Celite column (2 x 4 cm) and the solid was washed with several portions of MeCN. Evaporation of the combined filtrate and washings, and flash chromatography of the residue over silica gel (2 x 18 cm), using 4:100 MeOH-CH_2Cl_2, gave disulfide **43.6f** (44.4 mg, 58%) as a thick oil: $[\alpha]^{20}_D$ -47.7°; FTIR ($CDCl_3$ cast) 3300, 1665, 1608 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.58 (br s, 1 H), 2.33 (br s, 1 H), 2.85 (d, J = 6.2 Hz, 1 H), 3.20-3.23 (m, 1 H), 3.37-3.41 (m, 1 H), 3.57-3.61 (m, 1 H), 3.71-3.81 (m containing a singlet at δ 3.76, 4 H in all), 3.86-4.10 (m, 2 H), 4.16-4.43 (m, 4 H), 6.76-6.79 (m, 3 H), 6.96-7.03 (m, 2 H), 7.12-7.38 (m, 10 H), 8.14 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 32.2 (t′), 43.1 (t′), 43.4 (t′), 55.2 (q′), 59.7 (d′), 60.4 (d′), 63.4 (d′), 70.8 (t′), 72.7 (t′), 72.8 (t′), 73.0 (t′), 114.0 (d′), 114.1 (d′), 114.3 (d′), 127.4 (d′), 127.5 (d′), 127.7 (d′), 127.8 (d′), 127.9 (d′), 128.4 (d′), 128.5 (d′), 128.6 (d′), 128.7 (s′), 132.0 (s′), 137.4 (s′), 138.0 (s′), 159.2 (s′), 168.8 (s′), 172.9 (s′); exact mass m/z calcd for $C_{56}H_{65}N_6O_8S_2$ 1013.4305 (M + H), found 1013.4301.

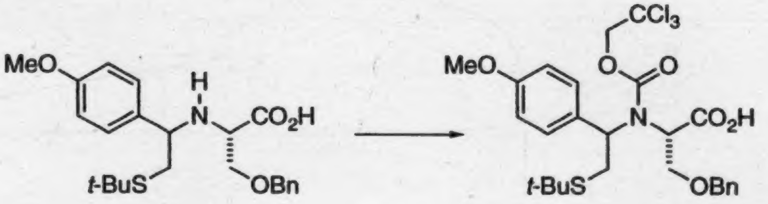
(2*S*)-3-Benzyloxy-2-[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethylamino]propionic Acid (44.1s).



43.1s **44.1s**

$Me_3SiOSO_2CF_3$ (413 μL, 2.28 mmol) was added dropwise to a stirred and cooled (0 °C) solution of ester **43.1s** (539 mg, 1.14 mmol) in dry CH_2Cl_2 (8.5 mL). Stirring was continued for 6 h without recharging the cold bath and the mixture was applied directly to a silica gel column (2 x 15 cm). Flash chromatography, using 4:100 MeOH-CH_2Cl_2, gave **44.1s** (403 mg, 84%) as a pale brown resinous solid: $[\alpha]^{20}_D$ +3.1° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.23 (s, 9 H), 3.00-3.05 (m, 1 H), 3.16-3.21 (m, 1 H), 3.49-3.50 (m, 1 H), 3.72 (s, 3 H), 3.81-3.90 (m, 2 H), 4.15-4.18 (m, 1 H), 4.47 (ABq, Δv_{AB} = 80.5 Hz, J_{AB} = 11.7 Hz, 2 H), 6.80-6.82 (m, 2 H), 7.20-7.29 (m, 7 H), 7.40 -8.00 (br s, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 30.8 (q′), 32.9 (s′), 43.4 (t′), 55.2 (q′), 58.9 (d′), 61.9 (d′), 67.1 (t′), 73.5 (t′), 114.6 (d′), 126.4 (s′), 127.9 (d′), 128.1 (d′), 128.5 (d′), 129.5 (d′), 137.2 (s′), 160.3 (s′), 169.9 (s′); exact mass *m/z* calcd for $C_{23}H_{32}NO_4S$ (M + H) 418.2046, found 418.2043.

3-Benzyloxy-2-[[2-tert-butylsulfanyl-1-(4-methoxyphenyl)ethyl](2,2,2-trichloroethoxycarbonyl)-amino]propionic Acid (44.2s).

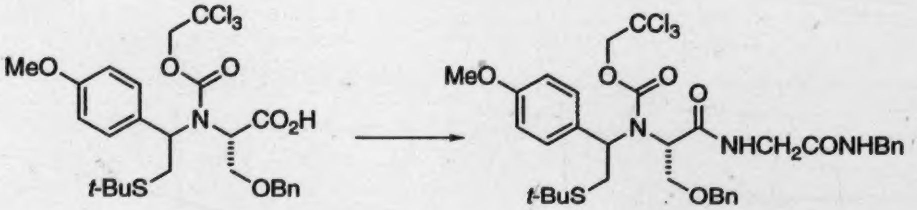


44.1s **44.2s**

A solution of Cl_3CCH_2OCOCl (300 μL, 2.17 mmol) in dioxane (1 mL) and 0.5 N NaOH (0.56 mL) were added simultaneously by syringe pump over 4.5 h to a stirred and cooled (0 °C) solution of **44.1s** (403 mg, 1.08 mmol) in 1 N NaOH (1.30 mL). When addition was complete the cold bath was removed and stirring was continued for 14 h. The mixture was diluted with water (5 mL), adjusted to pH 3-4 with 1 N hydrochloric acid, and extracted with Et_2O (2 x 15 mL). The combined extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 25 cm), using 8:100 MeOH-CH_2Cl_2, gave **44.2s** (314 mg, 52%) as a pale yellow foam: $[\alpha]^{20}_D$ -12.1° (*c* 1.0, $CHCl_3$); FTIR ($CHCl_3$ cast) 1715 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) (mixture of rotamers) δ 1.21 (s, 1.26 H), 1.28 (s, 1.46 H), 1.30 (s, 2.30 H), 1.33 (s, 4.0 H), 2.98-3.26 (m, 2 H), 3.76 (s, 3 H), 3.81-3.88 (m, 1 H), 3.95-4.20 (m, 2 H), 4.46-4.98 (m, 2 H), 5.45 (t, *J* = 7.1 Hz, 1 H), 6.82-6.87 (m, 2 H), 7.03-7.06 (m, 1 H), 7.21-

7.33 (m, 6 H), 9.0 (br s, 1 H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 28.5 (t′), 29.2 (t′), 29.8 (t′), 30.4 (t′), 31.1 (q′), 42.9 (t′), 43.3 (t′), 43.4 (t′), 43.7 (t′), 55.4 (q′), 56.5 (d′), 57.0 (d′), 57.2 (d′), 60.7 (d′), 61.4 (d′), 61.6 (d′), 68.6 (t′), 68.9 (t′), 69.5 (t′), 70.0 (t′), 73.3 (t′), 73.8 (t′), 73.9 (t′), 75.4 (t′), 75.6 (t′), 75.8 (t′), 95.0 (s′), 95.5 (s′), 95.6 (s′), 114.0 (d′), 114.3 (d′), 114.4 (d′), 127.7 (d′), 128.0 (d′), 128.2 (s′), 128.3 (d′), 128.5 (d′), 128.6 (d′), 128.8 (d′), 129.3 (d′), 130.3 (d′), 130.4 (d′), 130.5 (d′), 131.0 (d′), 131.1 (d′), 137.2 (s′), 137.4 (s′), 137.7 (s′), 153.8 (s′), 154.0 (s′), 159.7 (s′), 159.8 (s′), 159.9 (s′), 174.0 (s′); exact mass *m/z* calcd for $C_{26}H_{32}Cl_3NNaO_6S$ (M + Na) 614.0908, found 614.0908.

[(2*S*)-1-[(Benzylcarbamoylmethyl)carbamoyl]-2-benzyloxyethyl][2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethyl]carbamic Acid 2,2,2-Trichloroethyl Ester (44.3s).

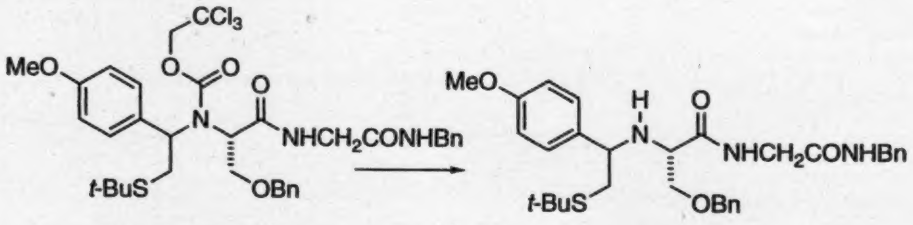


44.2s **44.3s**

$CF_3CO_2H.H_2NCH_2CONHBn$[45] (118 mg, 0.425 mmol) and Et_3N (172 μL, 1.23 mmol) were added with stirring to dry MeCN (0.85

mL). Acid **44.2s** (224 mg, 0.409 mmol) and then 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (136 mg, 0.425 mmol) were added. Stirring was continued for 12 h and the mixture was diluted with EtOAc (20 mL) and washed successively with 1 N hydrochloric acid (2 x 15 mL) and saturated aqueous $NaHCO_3$ (2 x 15 mL). The organic phase was dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 1:25 MeOH-CH_2Cl_2, gave **44.3s** (175 mg, 61%) as a white solid: $[\alpha]^{20}{}_D$ = -15.1° (*c* 1, $CHCl_3$); mp 46-51 °C; FTIR ($CDCl_3$ cast) 3349 (br), 1669, 1610 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) (mixture of rotamers) δ 1.17-1.48 (m, 9 H), 1.73 (s, 0.30 H), 2.48 (dd, *J* = 9.8, 5.9 Hz, 0.37 H), 2.91-3.23 (m, 2 H), 3.65-3.87 (m containing singlet at δ 3.78, 5 H in all), 3.87-4.60 (m, 7 H), 5.42-5.50 (m, 1 H), 6.27 (br s, 0.17 H), 6.65 (br s, 0.20 H), 6.66 (br s, 0.11 H), 6.78-6.90 (m, 2 H), 7.00-7.07 (m, 2 H), 7.17-7.35 (m, 10 H), 7.64 (t, *J* = 5.9 Hz, 0.49 H), 7.79 (br s, 0.17 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) (mixture of rotamers) δ 28.9 (t′), 29.6 (t′), 29.8 (t′), 30.3 (q′), 30.8 (q′), 30.9 (q′), 42.9 (t′), 43.2 (s′), 43.4 (t′), 43.6 (t′), 55.2 (q′), 56.3 (d′), 57.8 (d′), 59.7 (d′), 60.9 (d′), 67.7 (t′), 69.8 (t′), 72.9 (t′), 73.5 (t′), 74.9 (t′), 75.2 (t′), 95.1 (s′), 114.1 (d′), 114.4 (d′), 127.2 (d′), 127.3 (d′), 127.4 (d′), 127.6 (d′), 128.0 (d′), 128.1 (d′), 128.3 (d′), 128.4 (d′), 128.5 (s′), 128.7 (d′), 129.6 (d′), 129.7 (d′), 129.8 (d′), 136.7 (s′), 138.2 (s′), 153.7 (s′), 159.7 (s′), 168.7 (s′), 170.3 (s′); exact mass *m/z* calcd for $C_{35}H_{42}Cl_3N_3NaO_6S$ (M + Na) 760.1752, found 760.1753.

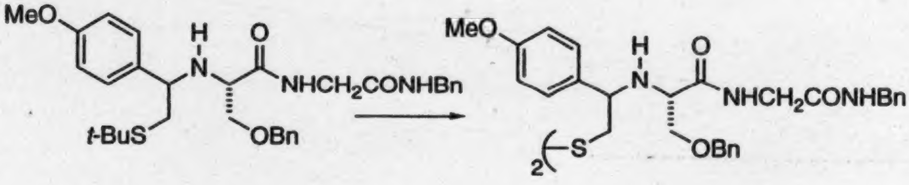
(2*S*)-*N*-(Benzylcarbamoylmethyl)-3-benzyloxy-2-[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethylamino]-propionamide (44.4s).



44.3s **44.4s**

Cd powder (787 mg, 7.00 mmol) was added in one portion to a stirred solution of **44.3s** (185 mg, 0.250 mmol) in 1:1 DMF-AcOH (5.4 mL). Stirring was continued for 4 h at room temperature, and the mixture was filtered through a Celite pad (2 x 4 cm), using EtOAc (50 mL). The combined filtrates and washings were washed with saturated aqueous $NaHCO_3$ (2 x 10 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 2:25 MeOH-CH_2Cl_2, gave **44.4s** (129 mg, 91%) as a pale yellow resin: $[\alpha]^{20}{}_D$ -4.1° (*c* 1, MeOH); FTIR ($CDCl_3$ cast) 3305, 1658 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) (mixture of rotamers) δ 1.29 (br s, 3 H), 1.31 (s, 6 H), 2.64-2.82 (m, 2 H), 3.04-3.06 (m, 1 H), 3.15-3.18 (m, 0.32 H), 3.34 (dd, *J* = 9.5, 3.8 Hz, 0.27 H), 3.50-3.56 (m, 0.34 H), 3.59-3.65 (m, 1 H), 3.69-3.78 (m containing singlet at δ 3.74, 4 H in all), 3.85-3.92 (m, 1 H), 4.03 (dd, *J* = 16.6, 6.6 Hz, 0.6 H), 4.13-4.21 (m, 1.4 H), 4.27-4.37 (m containing singlet at δ 4.36, 2.6 H in all), 6.60 (t, *J* = 5.2 Hz, 0.8 H), 6.73-6.85 (m, 2.4 H), 7.00-7.32 (m, 12 H), 7.99 (br s, 0.1 H), 8.10 (t, *J* = 5.9 Hz, 0.7 H), 8.44 (t, *J* = 6.3 Hz, 0.3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) (mixture of rotamers) δ 31.0 (q'), 31.1 (q'), 36.7 (s'), 36.8 (s'), 42.7 (t'), 43.0 (t'), 43.1 (t'), 43.3 (t'), 43.4 (t'), 55.2 (q'), 59.6 (d'), 59.7 (d'), 61.2 (d'), 62.2 (d'), 68.8 (d'), 71.0 (d'), 72.7 (d'), 73.3 (d'), 114.1 (d'), 127.3 (d'), 127.4 (d'), 127.5 (d'), 127.6 (d'), 127.7 (d'), 127.8 (d'), 128.2 (d') 128.4 (d'), 128.5 (d'), 133.9 (s'), 134.0 (s'), 137.4 (s'), 137.6 (s'), 138.0 (s'), 138.1 (s'), 159.1 (s'), 159.3 (s'), 168.9 (s'), 169.1 (s'), 173.1 (s'), 173.3 (s'); exact mass *m/z* calcd for $C_{32}H_{42}N_3O_4S$ 564.2890 (M + H), found 564.2893.

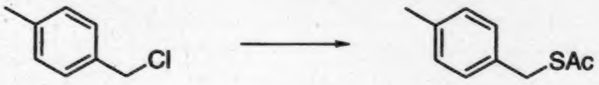
(2*S*)-*N*-(Benzylcarbamoylmethyl)-2-[2-[2-[1-[(benzylcarbamoylmethyl)carbamoyl]-2-benzyloxyethylamino]-2-(4-methoxyphenyl)ethyldisulfanyl]-1-(4-methoxyphenyl)ethylamino]-3-benzyloxypropionamide (44.5s).



44.4s **44.5s**

CF_3CO_2H (0.5 mL) was added to thioether **44.4s** (95.0 mg, 0.168 mmol) contained in a flask immersed in an ice-bath. The mixture was stirred and PhOMe (32 μL), followed by $Hg(OAc)_2$ (56.0 mg, 0.177 mmol) were added. Stirring was continued for 25 min and the solvent was evaporated. The residue was dissolved in MeCN (15 mL) and H_2S gas was bubbled through the solution for 2 min. The resulting black suspension was filtered through a tightly packed Celite column (2 x 4 cm) and the solid was washed with several portions of MeCN. Evaporation of the combined filtrate and washings, and flash chromatography of the residue over silica gel (2 x 18 cm), using 4:100 MeOH-CH_2Cl_2, gave **44.5s** (41.4 mg, 48%) as a pale brown oil: $[\alpha]^{20}_D$ +4.7° (*c* 1.0, $CHCl_3$); FTIR (CH_2Cl_2 cast) 3305, 1657 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) (mixture of disulfide and free thiol in a ratio of 3.1:1) δ 1.11-1.33 (m, 1 H), 1.47 (t, *J* = 8.3 Hz, 0.26 H), 2.54-2.82 (m, 2.47 H), 3.07 (t, *J* = 3.5 Hz, 0.78 H), 3.19 (dd, *J* = 6.0, 4.1 Hz, 0.17 H), 3.39 (dd, J = 9.5, 4.1 Hz, 0.21 H), 3.53-3.63 (m, 1.74 H), 3.71-3.74 (m containing a singlet at δ 3.74, 4.11 H in all), 3.97-4.04 (m, 1.15 H), 4.16-4.41 (m, 3.54 H), 6.62 (t, *J* = 5.7 Hz, 0.75 H), 6.72-6.84 (m, 2 H), 7.00-7.04 (m, 0.43 H), 7.11-7.35 (m, 9.7 H), 8.04 (t, *J* = 5.8 Hz, 0.69 H), 8.17 (t, *J* = 5.9 Hz, 0.22 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 32.2 (t'), 43.0 (t'), 43.2 (t'), 43.4 (t'), 55.2 (d'), 55.3 (q'), 59.4 (d'), 59.8 (q'), 63.5 (d'), 63.7 (d'), 68.5 (t'), 70.9 (t'), 72.8 (t'), 73.3 (t'), 73.4 (t'), 114.0 (d'), 114.3 (d'), 127.3 (d'), 127.4 (d'), 127.6 (d'), 127.7 (d'), 127.8 (d'), 127.9 (d'), 128.3 (d'), 128.4 (d'), 128.6 (d'), 128.7 (d'), 132.8 (s'), 133.0 (s'), 137.4 (s'), 137.5 (s'), 138.0 (s'), 159.2 (s'), 159.4 (s'), 168.8 (s'), 172.8 (s'), 172.9 (s'); exact mass *m/z* calcd for $C_{56}H_{65}N_6O_8S_2$ 1013.4305 (M + H), found 1013.4301.

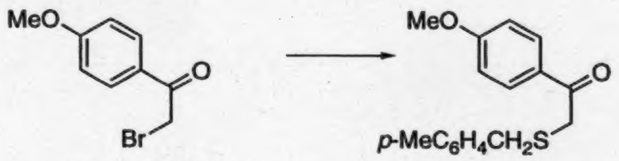
Thioacetic Acid *S*-(4-Methylbenzyl) Ester (45.2).



45.1 **45.2**

NaI (50 mg) was added to a stirred mixture of 4-methylbenzyl chloride (**45.1**) (1.27 mL, 9.59 mmol) and AcSK (1.21 g, 10.6 mmol) in dry DME (30 mL) (N_2 atmosphere). Stirring was continued for 11 h and the mixture was diluted with Et_2O (200 mL), washed with water (3 x 75 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 1:10 EtOAc-hexanes, gave **45.2** (1.56 g, 90%) as a pale yellow oil: FTIR ($CDCl_3$ cast) 1691 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.29 (s, 3 H), 2.32 (s, 3 H), 4.10 (s, 2 H), 7.11 (ABq, $\Delta\nu_{AB}$ = 21.5, J_{AB} = 8.0 Hz, 4 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.0 (q′), 30.3 (q′), 33.2 (t′), 128.6 (d′), 129.3 (d′), 134.5 (s′), 136.9 (s′), 195.2 (s′); exact mass *m/z* calcd for $C_{10}H_{12}OS$ 180.0608, found 180.0610.

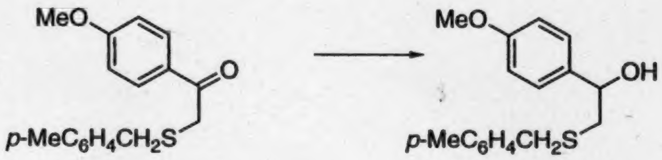
1-(4-Methoxyphenyl)-2-(4-methylbenzylsulfanyl)-ethanone (45.4).



3.2 **45.4**

BuLi (2.5 M in hexanes, 7.77 mL, 19.4 mmol) was added in one portion to a degassed (by passage of N_2), stirred and cooled (0 °C) solution of thioacetic acid *S*-(4-methylphenyl)methyl ester (**45.2**) (3.46 g, 19.2 mmol) in dry THF (60 mL). Stirring was continued for 25 min at 0 °C, and freshly prepared bromide **3.2** (4.40 g, 19.2 mmol) was then added in one portion. The cold bath was removed and the stirring was continued for 13 h. The mixture was diluted with Et_2O (300 mL), washed with water (3 x 100 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (3 x 20 cm), using 1:7 EtOAc-hexanes, gave **45.4** (3.44 g, 62%) as a white solid, which could not be freed of impurities by chromatography. The ^1H NMR spectrum showed the presence of the disulfide $(4\text{-}MeC_6H_4CH_2S)_2$. No other data were obtained as the desired product could not be freed of impurities. After reduction of the carbonyl (see below) a pure product was obtained.

1-(4-Methoxyphenyl)-2-(4-methylbenzylsulfanyl)-ethanol (45.5).



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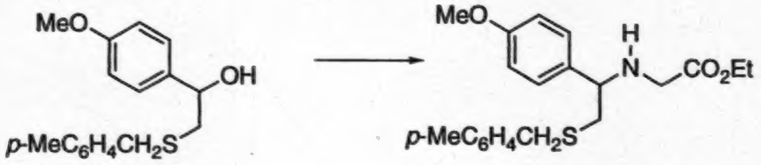
45.4 **45.5**

$NaBH_4$ (142 mg, 3.57 mmol) was added in three equal portions over 45 min to a stirred and cooled (0 °C) solution of **45.4** (341 mg, 1.19 mmol) in 1:1 MeOH-EtOAc (12 mL). After the addition the mixture was stirred at 0 °C for 0.5 h and the solvent was then evaporated. The residue was dissolved in 1:1 water-EtOAc (40 mL), the solution was stirred for 1 h and the organic phase was separated. The aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic phase and extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (1.5 x 15 cm), using 1:6 EtOAc-hexanes, gave **45.5** (235 mg, 68%) as a white solid: FTIR ($CDCl_3$ cast) 3438 (br) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.31 (s, 3 H), 2.62 (dd, J_{AB} = 13.9 Hz, J_{AX} = 9.2 Hz, 1 H), 2.73 (dd, J_{AB} = 13.8 Hz, J_{BX} = 3.8 Hz, 1 H), 2.76 (br s, 1 H), 3.66 (s, 2 H), 3.76 (s, 3 H), 4.59 (dd, J_{AX} = 9.2 Hz, J_{BX} = 2.5 Hz, 1 H), 6.82-6.84 (m, 2 H), 7.08-7.09 (m, 2 H), 7.16-7.21 (m, 4 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.1 (q′), 35.9 (t′), 40.9 (t′), 55.3 (q′), 71.4 (d′), 113.8 (d′), 126.9 (d′), 128.7 (d′), 129.2 (d′), 134.6 (s′), 134.7 (s′), 134.8

(s'), 159.1 (s'); exact mass *m/z* calcd for $C_{17}H_{20}O_2S$ 288.1184, found 288.1182.

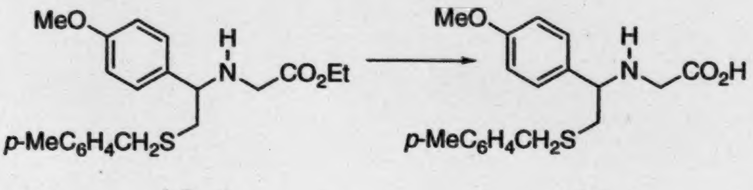
[1-(4-Methoxyphenyl)-2-(4-methylbenzylsulfanyl)-ethylamino]acetic Acid Ethyl Ester (46.1).



45.5 **46.1**

Me_3SiBr (99 μL, 0.74 mmol) was injected in one portion to a stirred and cooled (0 °C) solution of alcohol **45.5** (204 mg, 0.710 mmol) in dry CH_2Cl_2 (2 mL). Stirring was continued at 0 °C for 0.5 h and neat, freshly distilled (distilled under water pump vacuum) $H_2NCH_2CO_2Et$ (146 mg, 1.42 mmol) was added in one portion. The cold bath was removed and stirring was continued for 3 h. The mixture was adsorbed onto silica gel (2 g) from CH_2Cl_2. The solid was applied to the top of a column of silica gel (1.5 x 15 cm), and flash chromatography, using 1:6 EtOAc-hexanes, gave **46.1** (224 mg, 84%) as a colorless oil: FTIR ($CHCl_3$ cast) 3310 (br), 1735 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.23 (t, J = 7.1 Hz, 3 H), 2.30 (s, 3 H), 2.54 (dd, J_{AB} = 13.6 Hz, J_{AX} = 9.2 Hz, 1 H), 2.56-2.60 (br s, 1 H), 2.65 (dd, J_{AB} = 13.6 Hz, J_{BX} = 4.6 Hz, 1 H), 3.18 (ABq, Δv_{AB} = 52.9, J_{AB} = 17.5 Hz, 2 H), 3.63-3.73 (m, 3 H), 3.76 (s, 3 H), 4.15 (q, J = 7.1 Hz, 2 H), 6.80-6.85 (m, 2 H), 7.07-7.10 (m, 2 H), 7.15-7.20 (m, 4 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2 (q′), 21.1 (q′), 35.5 (t′), 39.3 (t′), 48.4 (t′), 55.2 (q′), 59.7 (d′), 60.5 (t′), 113.8 (d′), 128.2 (d′), 128.7 (d′), 129.0 (d′), 133.8 (s′), 134.9 (s′), 136.4 (s′), 158.9 (s′), 172.2 (s′); exact mass *m/z* calcd for $C_{21}H_{27}NO_3S$ 373.1712, found 373.1704.

[1-(4-Methoxyphenyl)-2-(4-methylbenzylsulfanyl)-ethylamino]acetic Acid Ethyl Ester (46.2).

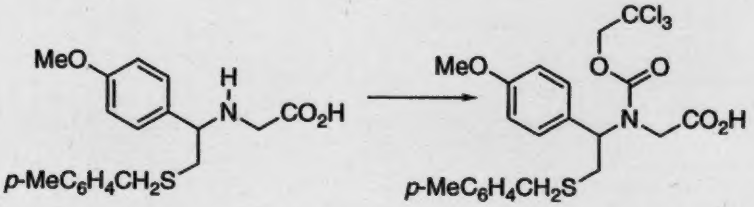


46.1 **46.2**

Aqueous NaOH (1N, 1.80 mL) was added to a stirred solution of ester **46.1** (424 mg, 0.880 mmol) in 1:1 water-dioxane (16 mL). Stirring was continued for 4 h, the mixture was acidified with 1 N hydrochloric acid, the solvent was evaporated and the residue was mixed with MeOH (5 mL), and adsorbed onto silica gel (3 g). The solid was applied to the top of a column of silica gel (1.5 x 15 cm), and flash chromatography, using 1:2:25 AcOH-MeOH-CH_2Cl_2, gave **46.2** (277 mg, 91%) as white solid: mp 165-169 °C; FTIR (microscope) 1609 cm^{-1}; ^{1}H NMR (CD_3CO_2D, 400 MHz) δ 1.53 (s, 1 H), 2.28 (s, 3 H), 2.97-3.02 (m, 1 H), 3.18-3.24 (m, 1 H), 3.63 (br s, 2 H), 3.68 (s, 2 H), 3.79 (s, 3 H), 4.36-4.40 (m, 1 H), 6.97

(d, J = 8.1 Hz, 2 H), 7.13 (ABq, Δv_{AB} = 32.7 Hz, J_{AB} = 7.8 Hz, 4 H), 7.40 (d, J = 8.2 Hz, 2 H); ^{13}C NMR (CD_3CO_2D, 100 MHz) δ 21.1 (q'), 34.8 (t'), 36.3 (t'), 55.8 (q'), 62.5 (d'), 115.7 (d'), 125.7 (s'), 130.0 (d'), 130.3 (d'), 131.1 (d'), 135.5 (s'), 138.0 (s'), 162.0 (s'); exact mass *m/z* calcd for $C_{19}H_{23}NNaO_3S$ 368.1296 (M + Na), found 368.1298.

[[1-(4-Methoxyphenyl)-2-(4-methylbenzylsulfanyl)-ethyl](2,2,2-trichloroethoxycarbonyl)amino]acetic Acid (46.3).

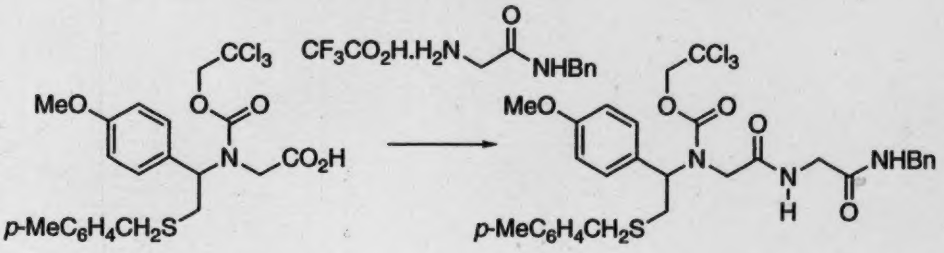


46.2 **46.3**

Neat Cl_3CCH_2OCOCl (220 µL, 1.61 mmol) and 1 N NaOH (210 µL) were added simultaneously by syringe over 4.5 h to a stirred and cooled (0 °C) suspension of **46.2** (277 mg, 0.805 mmol) in 1 N NaOH (0.97 mL) and dioxane (1 mL). When addition was complete the cold bath was removed and stirring was continued for 11 h, by which time all **46.2** had reacted. The acidic mixture was extracted with Et_2O and the combined extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 4:100 MeOH-CH_2Cl_2, gave **46.3** (314 mg, 74%) as an oil:

FTIR ($CDCl_3$ cast) 1716, 1611 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.30 (s, 3 H), 2.94-3.02 (m, 2 H), 3.69 (s, 2 H), 3.70-3.73 (m, 2 H), 3.77 (s, 3 H), 4.72-4.95 (m, 2 H), 5.42-5.49 (m, 1 H), 6.82-6.86 (m, 2 H), 7.07-7.10 (m, 2 H), 7.14-7.20 (m, 4 H); ^{13}C NMR ($CDCl_3$, 100 MHz) (mixture of rotamers) δ 21.1 (q′), 32.2 (t′), 32.8 (t′), 36.5 (t′), 44.6 (t′), 45.2 (t′), 55.2 (q′), 58.2 (d′), 58.5 (d′), 67.0 (t′), 75.5 (t′), 76.7 (t′), 95.2 (s′), 114.1 (d′), 128.6 (s′), 128.9 (d′), 129.2 (d′), 129.4 (d′), 129.5 (d′), 129.6 (d′), 134.6 (s′), 134.7 (s′), 136.7 (s′), 136.9 (s′), 154.3 (s′), 154.4 (s′), 159.6 (s′), 173.3 (s′), 173.8 (s′); exact mass *m/z* calcd for $C_{22}H_{24}Cl_3NNaO_5S$ 542.0338 (M + Na), found 542.0335.

[[(Benzylcarbamoylmethyl)carbamoyl]methyl][1-(4-methoxyphenyl)-2-(4-methylbenzylsulfanyl)ethyl]-carbamic Acid 2,2,2-Trichloroethyl Ester (46.4).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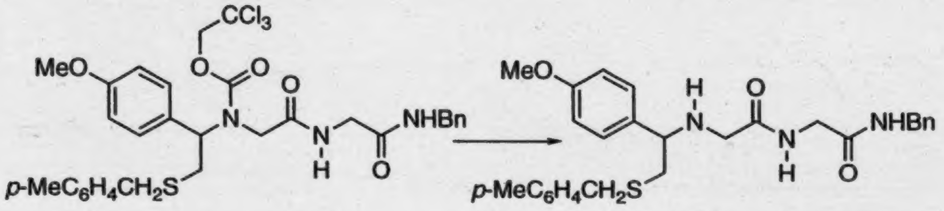
O
O
O
N
N
H
NHBn
O
p-MeC6H4CH2S

46.3 **46.4**

i-Pr_2NEt (115 μL, 0.664 mmol) was added to a stirred and cooled (0 °C) mixture of acid **46.3** (314 mg, 0.603 mmol) and $CF_3CO_2H.H_2NCH_2CONHBn$[45] (184 mg, 0.664 mmol) in dry CH_2Cl_2 (5 mL). After 5 min EDCI (127 mg, 0.664 mmol) was added, followed by DMAP (3 mg), and the mixture was stirred for 3.5 h without recharging the cold bath. The mixture was diluted with EtOAc (15 mL) and washed successively with 1 N hydrochloric acid (3 mL) and brine (2 x 5 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm), using 4:100 MeOH-CH_2Cl_2, gave **46.4** (296 mg, 73%) as a white foam: FTIR (CH_2Cl_2 cast) 3306 (br), 1693, 1659 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.29 (s, 3 H), 2.87-3.01 (m, 2 H), 3.47-3.97 (m containing a singlet at δ 3.74, 9 H in all), 4.29-4.45 (m, 2 H), 4.64-4.71 (m, 1 H), 5.38-5.42 (m, 1 H), 6.43 (br s, 1 H), 6.79 (d, J = 8.0 Hz, 2 H), 6.92 (br s, 1 H), 7.03-7.28 (m, 12 H); ^{13}C NMR ($CDCl_3$, 125 MHz) (mixture of rotamers) δ 21.1 (q′), 32.4 (t′), 32.7 (t′), 35.7 (t′), 35.9 (t′), 42.6 (t′), 43.0 (t′), 43.3 (t′), 43.8 (t′), 47.5 (t′), 47.9 (t′), 55.3 (q′), 57.9 (d′), 58.4 (d′), 75.0 (t′), 75.3 (t′), 94.9 (s′), 95.2 (s′), 114.3 (d′), 127.4 (d′), 127.5 (d′), 127.6 (d′), 127.7 (d′), 127.8 (d′), 128.4 (d′), 128.5 (d′), 128.6 (d′), 128.7 (d′), 128.8 (d′), 128.9 (s′), 129.2 (d′), 129.3 (d′), 133.9 (s′), 134.1 (s′), 136.8 (s′), 137.1 (s′), 137.7 (s′), 137.9 (s′), 154.7 (s′), 155.1 (s′), 159.5 (s′), 168.1 (s′), 168.4 (s′), 168.8 (s′), 169.3 (s′); exact mass *m/z* calcd for $C_{31}H_{34}Cl_3N_3NaO_5S$ 688.1182 (M + Na), found 688.1183.

N-(Benzylcarbamoylmethyl)-2-[1-(4-methoxyphenyl)-2-(4-methylbenzylsulfanyl)ethylamino]acetamide (46.5).



46.4 **46.5**

Cd powder (1.50 g, 13.3 mmol) was added in one portion to a stirred solution of **46.4** (296 mg, 0.444 mmol) in 1:1 DMF-AcOH (9.6 mL). Stirring was continued for 6 h at room temperature, and the mixture was filtered through a Celite pad (2 x 4 cm), using EtOAc (75 mL). The combined filtrates and washings were washed with saturated aqueous $NaHCO_3$ (2 x 15 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 x 15 cm), using 2:100 and then 1:10 MeOH-CH_2Cl_2, gave **46.5** (204 mg, 93%) as a pale yellow oil: FTIR ($CDCl_3$ cast) 3296, 1654 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.29 (s, 3 H), 2.62 (d, *J* = 6.6 Hz, 2 H), 2.90 (br s, 1 H), 3.04-3.13 (m, 2 H), 3.48 (br t, *J* = 6.7 Hz, 1 H), 3.61-3.75 (m containing a singlet at δ 3.72, 5 H in all), 3.86 (d, *J* = 5.0 Hz, 2 H), 4.36 (d, *J* = 5.6 Hz, 2 H), 6.76-6.81 (m, 3 H), 7.01-7.25 (m, 11 H), 7.73 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.1 (q'), 36.3 (t'), 38.9 (t'), 43.2 (t'), 43.4 (t'), 49.6 (t'), 55.2 (q'), 60.9 (d'), 114.1 (d'), 127.3 (d'), 127.5 (d'), 127.6 (), 127.8 (d'), 127.9 (d'), 128.0

(d'), 128.6 (d'), 128.7 (d'), 128.8 (d'), 129.2 (d'), 133.0 (s'), 124.5 (s'), 136.8 (s'), 137.9 (s'), 159.2 (s'), 168.6 (s'), 172.5 (s'); exact mass *m/z* calcd for $C_{28}H_{33}N_3NaO_3S$ (M + Na) 514.2140, found 514.2144.

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45 CF_3CO_2H (5 mL) was added slowly to a stirred and cooled (0 °C) solution of $BocHNCH_2CONHBn$ (2.65 g, 10.0 mmol) in dry CH_2Cl_2 (5 mL). Stirring was continued at 0 °C for 3.5 h, and the solvent was evaporated. The residue was mixed with Et_2O (50 mL) and the resulting precipitate was filtered, washed thoroughly with Et_2O and left under

oil pump vacuum to give $CF_3CO_2H.H_2NCH_2CONHBn$ (2.79 g, 100%) as a white solid: mp 154-157 °C; FTIR (microscope) 1672 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 3.70 (s, 2 H), 4.42 (s, 2 H), 7.24-7.32 (m, 5 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 41.5 (t'), 44.3 (t'), 128.5 (d'), 128.7 (d'), 129.6 (d'), 139.4 (s'), 167.1 (s').

46 Footnote 10 in: Cavelier, F.; Enjalbal, C. *Tetrahedron Lett.* **1996**, *37*, 5131-5134.

APPENDIX

University of Alberta Department of Chemistry
X-Ray Crystallography Laboratory

STRUCTURE REPORT

XCL Code: DLC0005

Date: 1 June 2000

Compound: 3,4-Dimethyl-7-hydroxy-1-aza-5-oxobicyclo[4.3.0]non-3-en-2-one

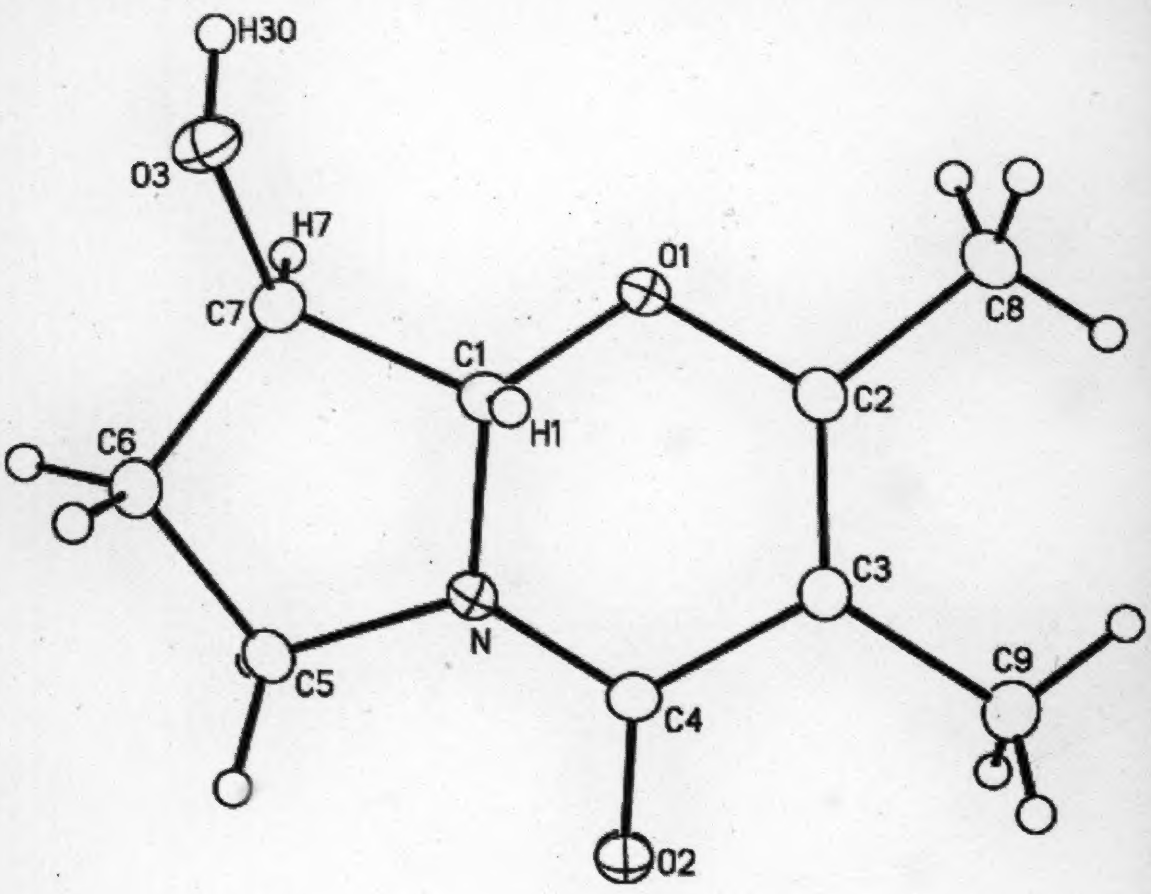
Formula: $C_9H_{13}NO_3$

Supervisor: D. L. J. Clive **Crystallographer:** R. McDonald

Figure Legends

Figure 1. Perspective view of the 3,4-dimethyl-7-hydroxy-1-aza-5-oxobicyclo[4.3.0]non-3-en-2-one molecule showing the atom labelling scheme. Non-hydrogen atoms are represented by Gaussian ellipsoids at the 20% probability level. Hydrogen atoms are shown with arbitrarily small thermal parameters.

Figure 2. Alternate view of the molecule.


H30
O3
H7
C7
O1
C1
H1
C2
C8
C6
C3
N
C5
C9
C4
O2

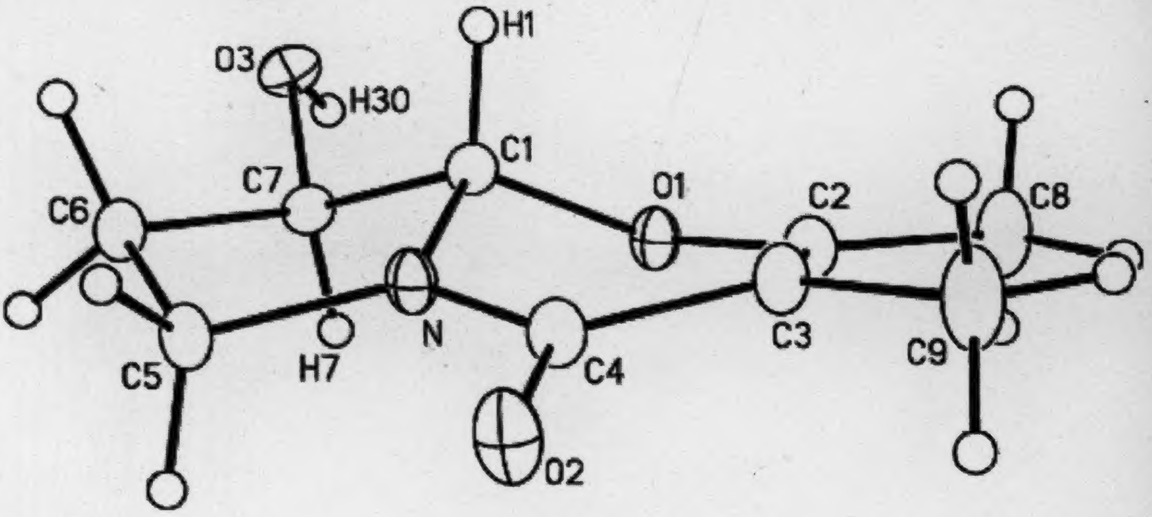

H1
O3
H30
C1
C7
C6
O1
C2
C8
N
C3
C9
H7
C5
C4
O2

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Table 1. Crystallographic Experimental Details

A. Crystal Data	
formula	$C_9H_{13}NO_3$
formula weight	183.20
crystal dimensions (mm)	0.38 × 0.24 × 0.16
crystal system	monoclinic
space group	$P2_1/c$ (No. 14)
unit cell parameters[a]	
a (Å)	9.4187 (9)
b (Å)	12.3627 (11)
c (Å)	8.0507 (8)
β (deg)	103.4161 (16)
V (Å^3)	911.85 (15)
Z	4
ρ_{calcd} (g cm^{-3})	1.335
μ (mm^{-1})	0.100
B. Data Collection and Refinement Conditions	
diffractometer	Bruker P4/RA/SMART 1000 CCD[b]
radiation (λ [Å])	graphite-monochromated Mo Kα (0.71073)
temperature (°C)	–80
scan type	ϕ rotations (0.3°) / ω scans (0.3°) (30 s exposures)
data collection 2θ limit (deg)	52.76
total data collected	4408 ($-9 \le h \le 11$, $-15 \le k \le 9$, $-9 \le l \le 10$)
independent reflections	1864 ($R_{\text{int}} = 0.0373$)
number of observations (*NO*)	1520 [$F_o^2 \ge 2\sigma(F_o^2)$]
structure solution method	direct methods (*SHELXS–86*[c])
refinement method (*SHELXL–93*[d])	full-matrix least-squares on F^2
absorption correction method	multi-scan (*SADABS*)
range of transmission factors	0.9841–0.9629
data/restraints/parameters	1864 [$F_o^2 \ge -3\sigma(F_o^2)$] / 0 / 122
extinction coefficient (x)[e]	0.007 (3)
goodness-of-fit (S)[f]	1.071 [$F_o^2 \ge -3\sigma(F_o^2)$]
final R indices[g]	
R_1 [$F_o^2 \ge 2\sigma(F_o^2)$]	0.0413
wR_2 [$F_o^2 \ge -3\sigma(F_o^2)$]	0.1178
largest difference peak and hole	0.230 and –0.170 e Å^{-3}

[a] Obtained from least-squares refinement of 3398 reflections with $5.20° < 2\theta < 52.59°$.

[b] Programs for diffractometer operation, data collection, data reduction and absorption

correction were those supplied by Bruker.

(continued)

Table 1. Crystallographic Experimental Details (continued)

[c]Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467–473.

[d]Sheldrick, G. M. *SHELXL-93*. Program for crystal structure determination. University of Göttingen, Germany, 1993. Refinement on F_o^2 for all reflections (all of these having $F_o^2 \geq -3\sigma(F_o^2)$). Weighted *R*-factors wR_2 and all goodnesses of fit *S* are based on F_o^2; conventional *R*-factors R_1 are based on F_o, with F_o set to zero for negative F_o^2. The observed criterion of $F_o^2 > 2\sigma(F_o^2)$ is used only for calculating R_1, and is not relevant to the choice of reflections for refinement. *R*-factors based on F_o^2 are statistically about twice as large as those based on F_o, and *R*-factors based on ALL data will be even larger.

[e]$F_c^* = kF_c[1 + x\{0.001F_c^2\lambda^3/\sin(2\theta)\}]^{-1/4}$ where *k* is the overall scale factor.

[f]$S = [\Sigma w(F_o^2 - F_c^2)^2/(n-p)]^{1/2}$ (n = number of data; p = number of parameters varied; $w = [\sigma^2(F_o^2) + (0.0555P)^2 + 0.2082P]^{-1}$ where $P = [\mathrm{Max}(F_o^2, 0) + 2F_c^2]/3$).

[g]$R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^4)]^{1/2}$.

Table 2. Atomic Coordinates and Equivalent Isotropic Displacement Parameters

Atom	x	y	z	U_{eq}, Å^2
O1	-0.14967(11)	0.31754(8)	0.18050(14)	0.0328(3)*
O2	-0.26807(13)	0.00931(9)	0.20594(18)	0.0487(4)*
O3	0.17314(12)	0.32259(9)	0.11980(14)	0.0378(3)*
N	-0.09580(13)	0.13154(10)	0.17938(17)	0.0321(3)*
C1	-0.06177(16)	0.23782(11)	0.12476(19)	0.0287(3)*
C2	-0.29631(17)	0.29216(12)	0.1380(2)	0.0348(4)*
C3	-0.34315(17)	0.18956(12)	0.1199(2)	0.0359(4)*
C4	-0.23423(17)	0.10175(12)	0.1690(2)	0.0338(4)*
C5	0.03382(16)	0.06422(12)	0.2431(2)	0.0353(4)*
C6	0.15791(17)	0.13562(13)	0.2126(2)	0.0352(4)*
C7	0.10003(16)	0.25139(12)	0.20935(19)	0.0302(4)*
C8	-0.3866(2)	0.39223(14)	0.1271(3)	0.0541(5)*
C9	-0.50164(19)	0.15730(15)	0.0775(3)	0.0570(6)*

Anisotropically-refined atoms are marked with an asterisk (*). The form of the anisotropic displacement parameter is: $\exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2klb^*c^*U_{23} + 2hla^*c^*U_{13} + 2hka^*b^*U_{12})]$.

Table 3. Selected Interatomic Distances (Å)

Atom1	Atom2	Distance	Atom1	Atom2	Distance
O1	C1	1.4250(17)	C1	C7	1.527(2)
O1	C2	1.3798(19)	C2	C3	1.340(2)
O2	C4	1.2408(18)	C2	C8	1.492(2)
O2	H3O^a	1.91†	C3	C4	1.483(2)
O3	C7	1.4139(18)	C3	C9	1.506(2)
N	C1	1.4449(18)	C5	C6	1.529(2)
N	C4	1.339(2)	C6	C7	1.530(2)
N	C5	1.4682(19)			

†Nonbonded distance. ^{a}At $\bar{x}$, $-^1/_2-y$, $^1/_2-z$.

Table 4. Selected Interatomic Angles (deg)

Atom1	Atom2	Atom3	Angle	Atom1	Atom2	Atom3	Angle
C1	O1	C2	112.70(11)	C2	C3	C9	124.02(15)
C1	N	C4	120.85(12)	C4	C3	C9	117.04(14)
C1	N	C5	113.37(12)	O2	C4	N	122.62(14)
C4	N	C5	125.78(13)	O2	C4	C3	122.53(14)
O1	C1	N	110.31(12)	N	C4	C3	114.76(13)
O1	C1	C7	112.09(11)	N	C5	C6	102.78(12)
N	C1	C7	103.47(11)	C5	C6	C7	105.09(12)
O1	C2	C3	121.85(14)	O3	C7	C1	113.24(12)
O1	C2	C8	110.48(13)	O3	C7	C6	112.18(12)
C3	C2	C8	127.58(15)	C1	C7	C6	102.69(12)
C2	C3	C4	118.30(14)				

Table 5. Torsional Angles (deg)

Atom1	Atom2	Atom3	Atom4	Angle
C2	O1	C1	N	-50.93(16)
C2	O1	C1	C7	-165.66(12)
C1	O1	C2	C3	28.0(2)
C1	O1	C2	C8	-155.20(15)
C4	N	C1	O1	42.17(18)
C4	N	C1	C7	162.24(14)
C5	N	C1	O1	-138.13(13)
C5	N	C1	C7	-18.06(16)
C1	N	C4	O2	175.97(15)
C1	N	C4	C3	-7.5(2)
C5	N	C4	O2	-3.7(3)
C5	N	C4	C3	172.88(15)
C1	N	C5	C6	-3.84(17)
C4	N	C5	C6	175.84(15)
O1	C1	C7	O3	-87.90(15)
O1	C1	C7	C6	150.91(12)
N	C1	C7	O3	153.26(12)
N	C1	C7	C6	32.07(15)
O1	C2	C3	C4	8.1(3)
O1	C2	C3	C9	178.63(17)
C8	C2	C3	C4	-168.19(18)
C8	C2	C3	C9	2.4(3)
C2	C3	C4	O2	157.80(17)
C2	C3	C4	N	-18.8(2)
C9	C3	C4	O2	-13.4(3)
C9	C3	C4	N	169.99(16)
N	C5	C6	C7	24.30(16)
C5	C6	C7	O3	-157.11(13)
C5	C6	C7	C1	-35.20(16)

Table 6. Anisotropic Displacement Parameters (U_{ij}, Å^2)

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O1	0.0291(6)	0.0231(5)	0.0464(7)	-0.0011(5)	0.0090(5)	0.0006(4)
O2	0.0365(7)	0.0268(6)	0.0813(10)	0.0077(6)	0.0108(6)	-0.0036(5)
O3	0.0407(7)	0.0363(6)	0.0398(7)	-0.0031(5)	0.0164(5)	-0.0111(5)
N	0.0280(7)	0.0231(6)	0.0448(8)	0.0023(6)	0.0081(5)	0.0012(5)
C1	0.0311(8)	0.0248(7)	0.0310(8)	-0.0001(6)	0.0089(6)	0.0001(6)
C2	0.0302(8)	0.0298(8)	0.0442(9)	0.0019(7)	0.0082(7)	0.0023(6)
C3	0.0277(8)	0.0306(8)	0.0493(10)	0.0011(7)	0.0089(7)	0.0007(6)
C4	0.0311(8)	0.0256(8)	0.0443(9)	-0.0009(7)	0.0076(7)	-0.0019(6)
C5	0.0317(9)	0.0279(8)	0.0453(10)	0.0013(7)	0.0074(7)	0.0046(6)
C6	0.0286(8)	0.0342(8)	0.0427(9)	0.0008(7)	0.0084(6)	0.0030(6)
C7	0.0300(8)	0.0311(8)	0.0307(8)	0.0000(6)	0.0095(6)	-0.0022(6)
C8	0.0382(10)	0.0331(9)	0.0915(16)	0.0031(9)	0.0162(10)	0.0078(7)
C9	0.0297(9)	0.0412(10)	0.0981(17)	0.0013(10)	0.0107(10)	-0.0015(7)

The form of the anisotropic displacement parameter is:

$$\exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2klb^*c^*U_{23} + 2hla^*c^*U_{13} + 2hka^*b^*U_{12})]$$

Table 7. Derived Atomic Coordinates and Displacement Parameters for Hydrogen Atoms

Atom	x	y	z	U_{eq}, Å^2
H3O	0.1942	0.3800	0.1759	0.057
H1	-0.0772	0.2400	-0.0023	0.034
H5A	0.0280	-0.0044	0.1785	0.042
H5B	0.0462	0.0477	0.3660	0.042
H6A	0.1821	0.1173	0.1027	0.042
H6B	0.2465	0.1265	0.3057	0.042
H7	0.1109	0.2779	0.3290	0.036
H8A	-0.4894	0.3724	0.1130	0.065
H8B	-0.3752	0.4355	0.0290	0.065
H8C	-0.3543	0.4345	0.2320	0.065
H9A	-0.5627	0.2224	0.0600	0.068
H9B	-0.5226	0.1149	0.1718	0.068
H9C	-0.5227	0.1137	-0.0270	0.068